EFFECTS OF METHAMPHETAMINE, IPRINDOLE AND PHENCYCLIDINE ON THE SEROTONERGIC SYSTEM OF THE RAT BRAIN

by

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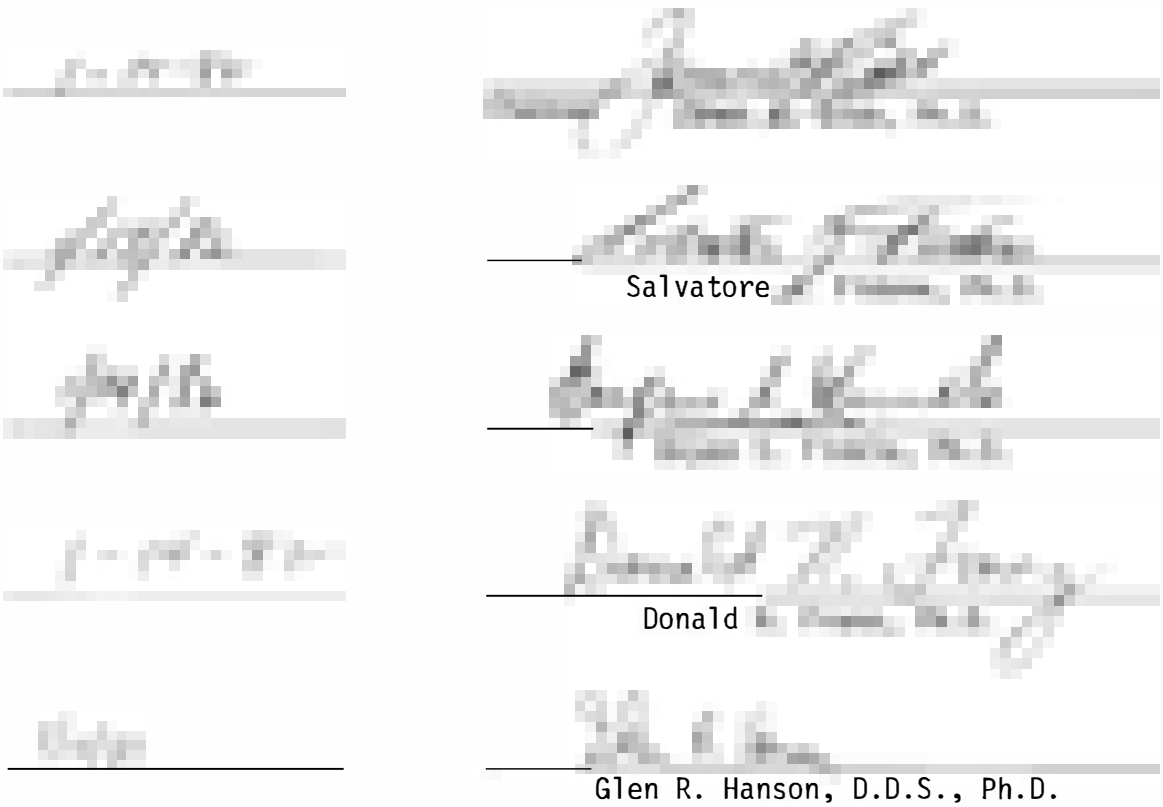

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ABSTRACT

The effects of administering methamphetamine to iprindole-treated rats on serotonergic metabolism in the cerebral cortex, neostriatum and hypothalamus and on dopamine metabolism in the neostriatum have been investigated. Methamphetamine (17.5 mg/kg, i.p.) was administered two hours after iprindole (10 mg/kg, i.p.). Three and 7 days after injection significant decreases ($p < 0.05$) were seen in tryptophan hydroxylase (TPH) activity, serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in all brain regions examined. A significant decrease in TPH activity was observed after 1 day; however, 5-HT and 5-HIAA concentrations were not significantly different from saline controls. After 14 days, all parameters were decreased with the exception of hypothalamic TPH activity, which was not significantly different from saline control. Neostriatal tyrosine hydroxylase (TH) activity and dopamine (DA) concentrations were significantly depressed at all time points examined. Methamphetamine (17.5 mg/kg i.p.) administered to iprindole-treated rats, was required to produce a significant decrease in these parameters of the serotonergic and dopaminergic systems. Significant decreases were observed using doses of iprindole of 5 mg/kg and methamphetamine of 17.5 mg/kg. Iprindole alone produced a significant increase in TPH activity (to 138% of control) in the cerebral cortex after 1 day. After 3 days, cortical TPH activity had fallen to 82% of control, whereas 5-HT and 5-HIAA were significantly increased over control. No effects were seen in the hippocampus, hypothalamus

or neostriatum. Cloimipramine, amitriptyline and chlorpromazine also produced significant increases in cortical TPH activity 1 day after an injection of 10 mg/kg. This evidence suggests that, in the presence of a metabolic inhibitor, methamphetamine has neurotoxic effects similar to the halogenated amphetamines on the serotonergic system. These drugs produce a long-lasting decrease in TPH activity and 5-HT concentrations. Secondly, administration of iprindole alone, and possibly other antidepressant drugs, produces changes in TPH activity that reflect an ability to alter 5-HT biosynthesis independent of brain tryptophan concentrations.

Phencyclidine (PCP) was found to have little effect on the neostriatal serotonergic system; the only significant changes being an increase in 5-HIAA concentrations after 15 and 30 minutes. Cerebellar GAD activity was decreased 6 and 12 hours after 4 injections of PCP (10 mg/kg, i.p.) over 12 hours. This decrease was observed at a dose of 5 mg/kg. No changes were seen after either acute or chronic administration of the drug. These changes in cerebellar GAD activity may account for the convulsant activity of this abused drug.

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INTRODUCTION

The proliferation of data implicating a role for the catecholamines and indoleamines in the mediation of symptoms associated with schizophrenia has increased attention to developing animal models for this naturally occurring human disorder. Since these models may represent a simplified form of the complex human phenomena, they provide a method for the preclinical evaluation of drug treatments. It must be remembered that, at best, animal models reflect only a limited subset of the symptoms associated with schizophrenia in humans.

Presently, the most widely-used animal model for schizophrenia deals with the behavioral changes induced by catecholamine stimulants and by amphetamines in particular. It is well known that drugs such as amphetamine, methylphenidate, or L-dopa exacerbate the psychotic symptoms of a subset of schizophrenics (Angrist, Rotrosen and Gershon, 1980 and Janowsky and Davis, 1976). Moreover, a high dose of amphetamine or chronic abuse of this stimulant elicits psychotic symptoms among nonschizophrenic individuals (Segal and Janowsky, 1978 and Snyder, 1973), and in the absence of a prior drug history these are often misdiagnosed as paranoid schizophrenics. The behavioral symptoms are distinguished by repetitive and compulsive behavior, hallucinations (auditory, visual and tactile), and delusions of persecution (Utena, 1966; Kramer, Fischman and Littlefield, 1967 and Hofman, 1975). "Amphetamine psychosis" has a duration of approximately one week. Residual behaviors have been reported to exist for several months (Kramer et al.,

1967) to a year (Utena, 1966) after drug use. These include a loss of initiative and ability to concentrate, impairment of memory and apathy.

Among non-human subjects, stimulant treatment elicits behaviors thought to reflect those observed among human drug abusers. Most importantly, these drugs cause stereotyped behaviors such as continuous sniffing, gnawing, grooming, and dyskinetic motor movements (Segal, 1975). Since these behaviors are exacerbated by repeated drug administration and antagonized by drugs that have antipsychotic properties (Groves and Rebec, 1976), the amphetamine-induced stereotypies have been taken as an approximate comparison of human psychotic disorders.

A consideration in evaluating the amphetamine model of schizophrenia is the possible neurochemical interactions that subserve the syndrome. Amphetamine (and methamphetamine) produce neurochemical changes in the noradrenergic, dopaminergic and serotonergic systems of the rat brain.

Koda and Gibb (1973), Buening and Gibb (1974) and Kogan, Nichols and Gibb (1976) showed that multiple toxic doses of methamphetamine caused a decrease in tyrosine hydroxylase (TH) activity, and dopamine (DA), and norepinephrine (NE) concentrations in the rat neostriatum. Morgan and Gibb (1980) showed that similar doses also decreased TH activity, and DA and NE concentrations in the olfactory tubercle but not in the other extrastriatal dopaminergic nuclei (nucleus accumbens and median eminence) examined. Furthermore, neostriatal and olfactory tubercle TH activities were significantly decreased up to 30 days after treatment with methamphetamine.

The consequence of the administration of single doses of amphetamine on DA metabolism are dose-dependent. Kuczenski (1980) has

suggested that the following sequence of neuronal and biochemical events occurs in the nigrostriatal DA pathway with increasing doses (0.5 to greater than 2.0 mg/kg) of amphetamine:

1) facilitated release of DA from striatal nerve endings with concomitant activation of DA synthesis;

2) a release of DA from nigral DA dendrites leading to inhibition of firing in a subpopulation of nigro-striatal neurons and accumulation of striatal DA;

3) activation of a striato-nigral feedback pathway leading to inhibition of DA neuronal activity and at high doses;

4) release of DA by amphetamine, independent of impulse flow, coupled with a blockade of DA reuptake and metabolism through extraneuronal monoamine oxidase.

Steranka and Sanders-Bush (1980) and Ellison, Eison, Huberman and Daniel (1978) have used either subcutaneously implanted minipumps or silicone pellets to examine the effects of continuously administered amphetamine. Ellison et al. (1978) found that TH activity was decreased in the neostriatum several days after implantation; enzyme activity had not returned to control values 110 days after pellet removal. TH activity was not decreased at 110 days if a dose of amphetamine equivalent to that delivered from the silicone pellet was given by daily injection. Steranka and Sanders-Bush (1980) showed that 2 weeks after infusion of amphetamine for 3 days whole brain DA, but not 5-hydroxytryptamine (5-HT) or NE, was decreased. These findings were in contrast to the results obtained after infusion of p-chloroamphetamine; which decreased brain 5-HT 2 weeks after infusion. Bakhit, Peat and Gibb

(in press) found the neostriatal enzyme activity was decreased 110 days after repeated injections of methamphetamine.

Since an early report by Knapp, Mandell and Geyer (1974) of the effects of amphetamine on the serotonergic system in the rat brain, increasing attention has been paid to the effects of amphetamine and methamphetamine on this neurotransmitter system. Knapp et al. (1974) found that the acute administration of d- and l-amphetamine, methamphetamine and p-chloroamphetamine reduced the conversion of tryptophan (Try) to 5-HT in striatal synaptosomes. Tryptophan hydroxylase (TPH) activity in the lateral midbrain was decreased; however, activity in the medial midbrain was unchanged. None of the amphetamines nor two major metabolites of amphetamine had a demonstrable effect on TPH activity in vitro. Hotchkiss and Gibb (1980) extended this work and showed that 12 hours after 4 doses of methamphetamine (15 mg/kg), neostriatal and hippocampal TPH activity were decreased to approximately 10% of control and this depression persisted for at least 30 days. Fluoxetine, a selective 5-HT uptake inhibitor (Wong, Horng, Bymaster, Hauser and Molloy, 1974), blocked this decrease in TPH activity, but not the concomitant depression of neostriatal TH activity, suggesting that uptake of methamphetamine into the nerve terminal is required for its toxic effect on 5-HT synthesis. It has also been observed that fluoxetine prevents the neurotoxicity resulting from p-chloroamphetamine administration (Fuller, Perry and Molloy, 1975).

Trulson and Jacobs (1979 a, 1979 b and 1980), Ricaurte, Schuster and Seiden (1980) and Bakhit et al. (in press) have all reported effects of chronic methamphetamine or amphetamine on the serotonergic system. Trulson and Jacobs (1980) and Bakhit et al. (in press) found, in

agreement with Hotchkiss and Gibb (1980), that TPH activity was decreased after multiple doses of amphetamines. All groups reported that 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were also decreased. In a similar fashion to the dopaminergic system, recovery in the serotonergic system was incomplete at 110 days after 4 repeated doses of methamphetamine (Bakhit et al., in press). Certain brain regions are also more sensitive to the effects of amphetamines, with cerebral cortex, hippocampus and striata being amongst the most sensitive and the hypothalamus being relatively resistant. This regional selectivity closely parallels the regional distribution of amphetamine as reported by Eison, Ellison and Eison (1981). Both Bakhit et al. (in press) and Ricaurte et al. (1980) have suggested that the serotonergic systems of the rat brain are more sensitive than the dopaminergic ones to the apparent neurotoxic actions of methamphetamine.

The acute effects of methamphetamine (or amphetamine) on the serotonergic system have not been as extensively studied. Recent work in our laboratory has shown that cortical and neostriatal TPH activity are decreased 15 minutes after a single dose of methamphetamine (10 mg/kg), whereas 5-HT concentrations are depressed at 30 minutes. No significant changes were seen in either neostriatal TH activity or DA concentrations. This work suggests that the primary action of methamphetamine is to cause inhibition of TPH, and that the depletion of 5-HT is secondary to this inhibition. This hypothesis is supported by the work of Trulson and Jacobs (1980) using the chronic administration of amphetamine.

It appears from the previous studies with amphetamine or methamphetamine that both the dose of the drug and its persistence at the

site of action are important for the apparent neurotoxic effects of these amphetamines. Fuller and Hemrick-Lueke (1980) have shown that neostriatal DA concentrations are decreased 7 days after the administration of amphetamine to iprindole-treated rats. Iprindole, an antidepressant drug, inhibits the hepatic metabolism and markedly increases the brain half-life of amphetamine in the rat (Freeman and Sulser, 1972). Amfonelic acid, a DA uptake blocker (Shore, 1976), inhibited this effect of amphetamine in iprindole-treated rats which suggests that uptake of drug into the dopamine neuron is required for the neurotoxic effect. The effects of combined administration of methamphetamine and iprindole on the serotonergic system in the rat brain have not been reported. If long-lasting effects on this neurotransmitter system are observed, the neurotoxicity of this stimulant may be comparable to that observed with p-chloroamphetamine and fenfluramine.

Sanders-Bush, Busing and Sulser (1975) found that p-chloroamphetamine decreased 5-HT concentrations and TPH activity in several brain regions, with the hippocampus, cerebral cortex and neostriatum being particularly sensitive. As with methamphetamine, the hypothalamus was particularly resistant to the effects of p-chloroamphetamine: a depression of cerebral cortical TPH activity and 5-HT and 5-HIAA concentrations was still observed 4 months after a single injection. Fenfluramine (21 mg/kg), another halogenated amphetamine, decreased 5-HT concentrations and TPH activity after 1 day, although the effects were greatly diminished 14 days later. Sanders-Bush et al. (1975) also reported a marked reduction in the high-affinity uptake of 5-HT by synaptosomes, which persists for several weeks after a single dose of p-chloroamphetamine. Ricaurte et al. (1980) found a loss of 5-HT

synaptosomal uptake sites after both methamphetamine and p-chloroamphetamine administration.

The primary aim of the research described in this thesis was to evaluate the combined effects of methamphetamine and iprindole on the serotonergic system in discrete nuclei of the rat brain. TPH and TH activity, DA, Try, 5-HT and 5-HIAA concentrations were determined by methods described in the "Methods" section.

Recently, phencyclidine [1-(phenylcyclohexyl) piperidine or PCP] psychosis has been proposed as a drug model for acute schizophrenia (Allen and Young, 1978 and Meltzer and Stahl, 1976). PCP was originally employed as an anesthetic (Chen, Ensor, Russel and Bohner, 1959). Until recently it was used as a veterinary anesthetic. Because of its psychotomimetic properties, PCP is now widely abused for its mind-altering effects. Prolonged toxic reactions to PCP ingestion, leading in many cases to hospital admission for schizophrenia-like psychoses (Allen and Young, 1978, Liden, Lovejoy and Costello, 1975 and Showalter and Thornton, 1977) are not uncommon. These toxic psychoses may last for as long as several weeks; this effect is probably due to accumulation of the drug in adipose tissue and brain (Mishra, Pontani and Bartolomea, 1979).

The toxic psychosis produced by PCP is similar to acute schizophrenia in some respects such as its variable course and clinical picture, which may include catatonic, hebephrenic and paranoid features (Allen and Young, 1978). Because of these similarities, PCP psychosis has been proposed as a drug model for schizophrenia. While an understanding of the neuropharmacology of PCP has potential implications for

schizophrenia research and treatment, the mechanisms underlying this drug-induced psychosis are poorly understood.

In rodents, low doses of PCP produce central stimulation characterized by increased locomotor activity and stereotyped behavior, not unlike that caused by stimulants such as amphetamine (Sturgeon, Fessler and Meltzer, 1979). The effect of PCP on food-reinforced operant behavior is qualitatively similar to d-amphetamine (Wenger and Dews, 1976). PCP induces ipsilateral turning in rats with unilateral electrolytic or 6-hydroxydopamine-induced lesions in the nigrostriatal pathway, a finding consistent with an indirect rather than a direct DA agonist action (Fessler, Sturgeon and Meltzer, 1979). The fact that inhibition of DA synthesis with α-methylparatyrosine blocks this effect also suggests that PCP acts as an indirect DA agonist (Fessler et al., 1979).

Numerous reports indicate that PCP has important effects on central dopaminergic, noradrenergic, cholinergic, serotonergic, GABA-ergic and endogenous opiate transmitter systems (for review see Johnson 1978). Radioligand studies have indicated the existence of specific PCP receptors that may be relatively enriched in the hippocampus (Vincent, Karolovski, Geneste, Kamenka and Lazdunski, 1979 and Zukin and Zukin, 1979). Recent work with the monoaminergic system has focused upon the effects of PCP on the noradrenergic and dopaminergic systems.

Electrophysiological studies (Bickford, Palmer, Rice, Hoffer and Freedman, 1981 and Marwaha, Palmer, Woodward, Hoffer and Freedman, 1980) have concentrated upon the effects of PCP on noradrenergic transmission in the cerebellum and hippocampus. The results of the study on Purkinje neurons in the cerebellum (Marwaha et al., 1980) suggested that

PCP acts by a presynaptic mechanism involving the release of NE from intact functioning noradrenergic terminals. Smith, Taylor, Ho and Leelavathi (1980) and Doherty, Somonovic, So and Meltzer (1980) have reported the effects of PCP on neostriatal dopaminergic metabolism. Smith et al. (1980) showed that a single dose (10 mg/kg) decreased neostriatal TH activity 15 minutes after administration; recovery was noted at 45 minutes. Chronic administration also decreased TH activity after 30 doses. Doherty et al. (1980) examined the effects of PCP on neostriatal 3,4-dihydroxyphenylalanine (DOPA) accumulation, after L-aromatic acid decarboxylase inhibition, and concentrations of DA and its metabolites; 3,4-dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA). Their results suggested that the mechanism of action of PCP appears to be closely related to other non-amphetamine stimulants such as methylphenidate and amfonelic acid. In this regard, an early report by Smith, Meltzer, Arora and Davis, (1977) demonstrated that PCP was similar in potency to d-amphetamine and methylphenidate in inhibiting catecholamine ([^{3}H]-NE and [^{3}H]-DA) uptake in synaptosomal preparations. The same group also showed that PCP was considerably more potent than either amphetamine or methylphenidate in inhibiting [^{3}H]-5-HT uptake.

Although it is postulated that the dopaminergic and noradrenergic systems are both involved in the manifestation of the symptoms of schizophrenia; the evidence obtained using amphetamine (or methamphetamine) administration as a model for schizophrenia also suggests that the serotonergic system is involved. If this is so, then the animal model produced by PCP administration may also reflect changes in the serotonergic system. A secondary aim of the research described in this

thesis was to examine the effects of PCP on the serotonergic system in discrete nuclei of the rat brain, and to observe any neurochemical similarities between these and those of methamphetamine.

METHODS

Animals

Male, Sprague-Dawley rats weighing 200-300 gm were used for all experiments. The animals were housed 3 to 5 per cage with constant access to food and water. A 12-hour light-dark cycle (6:00 am - 6:00 pm) was maintained in the animal quarters.

Drug Administration

All drugs were administered in 0.9% saline by i.p injection. Saline was used to inject control animals. The following dosage schedules were used for the experiments:

1) Methamphetamine was administered 2 hours after a single injection of iprindole. The exact doses used are shown in the appropriate Table or Figure legends. Rats were sacrificed 1, 3, 7 and 14 days after the injection of methamphetamine to iprindole-treated rats.

2) For the experiments involving iprindole alone, animals were sacrificed 2, 6, 24 hours, 3 and 7 days after a single i.p. injection (10 mg/kg). For experiments involving other antidepressants, animals were sacrificed 24 hours after a single i.p. injection (10 mg/kg).

3) Experiments involving PCP were carried out using 3 different dosing schedules:

a) Rats were sacrificed at a number of time points between 15 and 120 minutes after a single injection of PCP (10 mg/kg).

b) Rats were sacrificed at a number of time points between 1 and 24 hours following 4 injections of PCP at 3-hour intervals over a period of 12 hours.

c) Rats were sacrificed 15 minutes and 24 hours after 30 daily injections of PCP (10 mg/kg).

Brain Dissection

All animals were sacrificed between 9:00 am and 4:00 pm to avoid diurnal rhythms in enzyme activities and neurotransmitter concentrations. Brains were rapidly removed and neostriata, cerebral cortex, hippocampus, hypothalamus and cerebellum dissected out and frozen separately on dry ice. Tissues were stored at -70° until analysis.

Tryptophan Hydroxylase Assay

Tissues were homogenized separately in 50 mM HEPES buffer (Sigma), pH 7.4, containing 0.2% Triton X-100 and 5 mM dithiothreitol, and centrifuged for 15 minutes at 19,000 X g. Duplicate 7.5-µl aliquots of the supernatant were assayed for TPH activity by the method of Ichiyama et al. (1970) as described by Sitaram and Lees (1978). The final concentrations of the reactants were as follows: HEPES, 50 mM, pH 7.4 at 25°C; L- (^{12}C) - tryptophan, 0.1 mM, L- (^{14}C) - tryptophan, 0.02 mM (10 nCi) and hog kidney L-aromatic acid decarboxylase in excess. The final reaction mixture (12.5 µl) was contained in a 6 x 50 mm silanized glass tube. A $^{14}CO_2$ trapping tube of the same size contained an 8 x 15-mm piece of filter paper wetted with 50 µl hyamine hydroxide (1.0 M in methanol; Sigma). The reaction tube and trapping tube were joined with a 5-cm piece of tightly fitting rubber tubing and incubated at 37°C for 30 minutes. The reaction was terminated by the injection of 100 µl of

5 N sulfuric acid into the reaction tube, and then incubated an additional 90 minutes at 37°C to complete the trapping of CO_2. The filter paper was then transferred to a scintillation vial and the trapping tube rinsed with 4 ml of scintillation cocktail. The combined rinse and filter paper were counted by liquid scintillation spectroscopy with a counting efficiency of 90-95%.

Tyrosine Hydroxylase and Tryptophan Hydroxylase Assays

Aliquots of the same neostriatal homogenate were analyzed concurrently for TH and TPH activities. Tissues were homogenized in 4 volumes of 50 mM HEPES buffer (Sigma), pH 7.4, containing 0.2% Triton X-100 and 5 mM dithiothreitol and centrifuged for 15 minutes at 19,000 X g. Duplicate 7.5-µl aliquots of the supernatant were added to 42.5 µl of double distilled water and assayed for TH activity according to the method of Nagatsu et al. (1964). TPH activity was determined as previously described.

Glutamic Acid Decarboxylase Assay

Glutamic acid decarboxylase (GAD) activity was determined by a modification of the method of Albers and Brady (1959). In brief, tissues were homogenized in 24 volumes 0.2% Triton X-100 containing 1 mg/ml bovine serum albumin (BSA) and 4 mM dithiothreitol. After centrifugation for 15 minutes at 19,000 X g, 10 µl of the supernatant were added to 10 µl reaction mixture containing (final concentration) 38 mM potassium phosphate buffer, pH 6.5; 12 mM sodium glutamate, pH 6.5; 0.2 mM pyridoxal phosphate; 1 mM potassium chloride; 0.1% Triton X-100; 0.05 mg/ml BSA; 2 mM dithiothreitol and 0.1 µCi 1-L-^{14}C glutamic acid. Labelled CO_2 was trapped on filter paper soaked with

hyamine hydroxide (1.0 M in methanol) and counted via liquid scintillation spectroscopy.

Determination of Indoleamines, Dopamine and Norepinephrine

In order to quantitate 5-HT, 5-HIAA, Try, DA and NE in discrete brain regions it was necessary to develop a high performance liquid chromatographic (HPLC) procedure using fluorometric detection (Peat and Gibb, submitted). All determinations were performed using a model 5020 solvent delivery system (Varian Instrument) with a loop injector (Valco Instrument Co.) and a model 650 fluorometer equipped with an 18-µl flow cell (Perkin Elmer Corp.). The excitation wavelength was set at 290 nm and the emission at 330 nm, the detector signal was recorded on a Linear recorder (10 mV input). An Ultrasphere-ODS reversed phase column (5 µm particle size range); 150 x 4.6 mm id (Altex Scientific) was used. The mobile phase was prepared with potassium phosphate monobasic (0.02 M) containing 1 gm per liter of heptane sulfonic acid sodium salt, adjusted to a pH of 3.3 using phosphoric acid, and a mixture of methanol: double distilled deionized water (3:2). Thirty-four percent of the organic modifier was used.

A stock solution of indoleamines and catecholamines in 0.1 M perchloric acid containing 4 mM sodium metabisulfite was prepared. This contained 0.1 mg/ml of 5-HT, 0.1 mg/ml of 5-HIAA, 0.5 mg/ml of Try, 0.25 mg/ml of DA and 0.1 mg/ml of NE. Aliquots (100 µl) of this stock solution were stored at -15°C and were used to prepare standard solutions as shown in Table 1.

TABLE 1

PREPARATION OF STANDARDS

(ng/µl)

Solution	Dilution	5-HT	5-HIAA	Try	DA	NE
A	1in 100 stock	1.0	1.0	5.0	2.5	1.0
B	1in 4A	0.25	0.25	1.25	0.625	0.25
C	1in 10A	0.1	0.1	0.5	0.25	0.1
D	1in 2C	0.05	0.05	0.25	0.125	0.05
E	1in 5A	0.2	0.2	1	0.5	0.2

Dilutions were made with the perchloric acid solution. Before use, all solutions were protected from light. Standards A-E must be made during each set of analyses.

Brain regions were homogenized in 0.1 M perchloric acid containing 4 mM sodium bisulfite. The amount of acid used for homogenization depended upon the amount of tissue available; for example 10 mg of neostriatum were homogenized in 100 µl of acid and cerebral cortex (50 mg) in 500 µl of solution. After homogenization, the extract was centrifuged at 19,000 x g for 15 minutes. Fifty µl of the supernatant were then injected via a 50-µl sample loop directly onto the HPLC column. All of the procedures, before injection, were carried out at 4°C and the extracts protected from light.

The concentration of each of the compounds in brain tissue was determined from the chromatographic peak heights. Calibration curves were constructed by injecting standard solutions B-E and plotting the amount of compound against peak heights.

Table 2 lists the retention volumes of the compounds examined using this system. Metabolites of dopamine and norepinephrine were not detectable under the fluorescent conditions chosen for the assay.

TABLE 2

RETENTION VOLUMES OF SOME INDOLEAMINES AND CATECHOLAMINES

Compound	Retention Volume (ml)
Tyrosine	3.2
5-Hydroxytryptophan	3.6
Norepinephrine	4.0
5-Hydroxyindoleacetic acid	6.0
N-Acetylserotonin	6.4
Dopamine	8.2
Tryptophan	14.0
5-Hydroxytryptamine	16.6
Melatonin	36.4

Sensitivity of the assay for 5-HT, 5-HIAA, NE, DA and Try was determined by injecting known amounts of each compound in perchloric acid solution. At excitation and emission wavelengths of 290 and 300 nm respectively, sensitivity was greatest for 5-HT and decreased in the following order: 5-HT > 5-HIAA > NE > DA > Try. The limits of sensitivities (signal to noise 3:1) were found to be 1.1 pmoles for 5-HT, 2.0 pmoles 5-HIAA, 3.0 pmoles NE, 4.9 pmoles DA and 6.1 pmoles Try. Linearity of the detector was found to be satisfactory for standards B through E (Table 1). Within-run precision studies for all five compounds gave a coefficient of variation of less than 8%.

RESULTS

Effects of Methamphetamine and Iprindole

Figures 1 to 3 illustrate the effects of consecutive doses of iprindole (10 mg/kg) and methamphetamine (17.5 mg/kg) on TPH activity, 5-HT and 5-HIAA concentrations at 4 time points (1, 3, 7 and 14 days) after injection in the cerebral cortex, neostriatum and hypothalamus. Previous studies (Bakhit et al., in press and Ricaurte et al., 1980) have shown that the cortex and neostriatum are particularly sensitive to the effect of methamphetamine whereas the hypothalamus is relatively resistant. Significant decreases were observed in TPH activity and 5-HT concentrations in the brain regions examined at 3 and 7 days with the maximum depression in enzyme activity and indoleamine concentrations seen 3 days after injection. Although neostriatal and cortical enzyme activities were decreased at 14 days (by 23 and 25% respectively), hypothalamic activity had returned to control values; this was in contrast to indoleamine concentrations which were still significantly depressed.

TPH activities were decreased in the cortex (by 44%), neostriatum (by 46%) and in the hypothalamus (by 44%) 1 day after the administration of methamphetamine to iprindole-treated rats. This was in contrast to indoleamine (5-HT and 5-HIAA) concentrations which were not significantly different from control values. Different effects were observed in the neostriatal dopaminergic system (Figure 4); TH activity and DA concentrations 1 day following treatment were decreased by 31% and 35%, respectively. In a similar fashion to the neostriatal serotonergic

FIG. 1 Effect of methamphetamine (METH) and iprindole (IPR) on TPH activity, 5-HT and 5-HIAA concentrations in the cerebral cortex at 1, 3, 7 and 14 days after injection. Rats were injected with either saline/METH (– –), IPR/saline (– · –) or IPR/METH (——). IPR (10 mg/kg) or saline were administered 2 hours before injection of METH (17.5 mg/kg) or saline. The number of animals per group were 4 or more. Brackets indicate ± S.E.M. Control values were 25.9 ± 1.1 nmoles Try oxidized/gm tissue/hr (n = 16), 0.21 ± 0.009 5-HT ng/mg tissue (n = 16) and 0.14 ± 0.006 5-HIAA ng/mg tissue (n = 16).

(*) $p < 0.05$ compared to saline/saline control

(**) $p < 0.005$ compared to saline/saline control

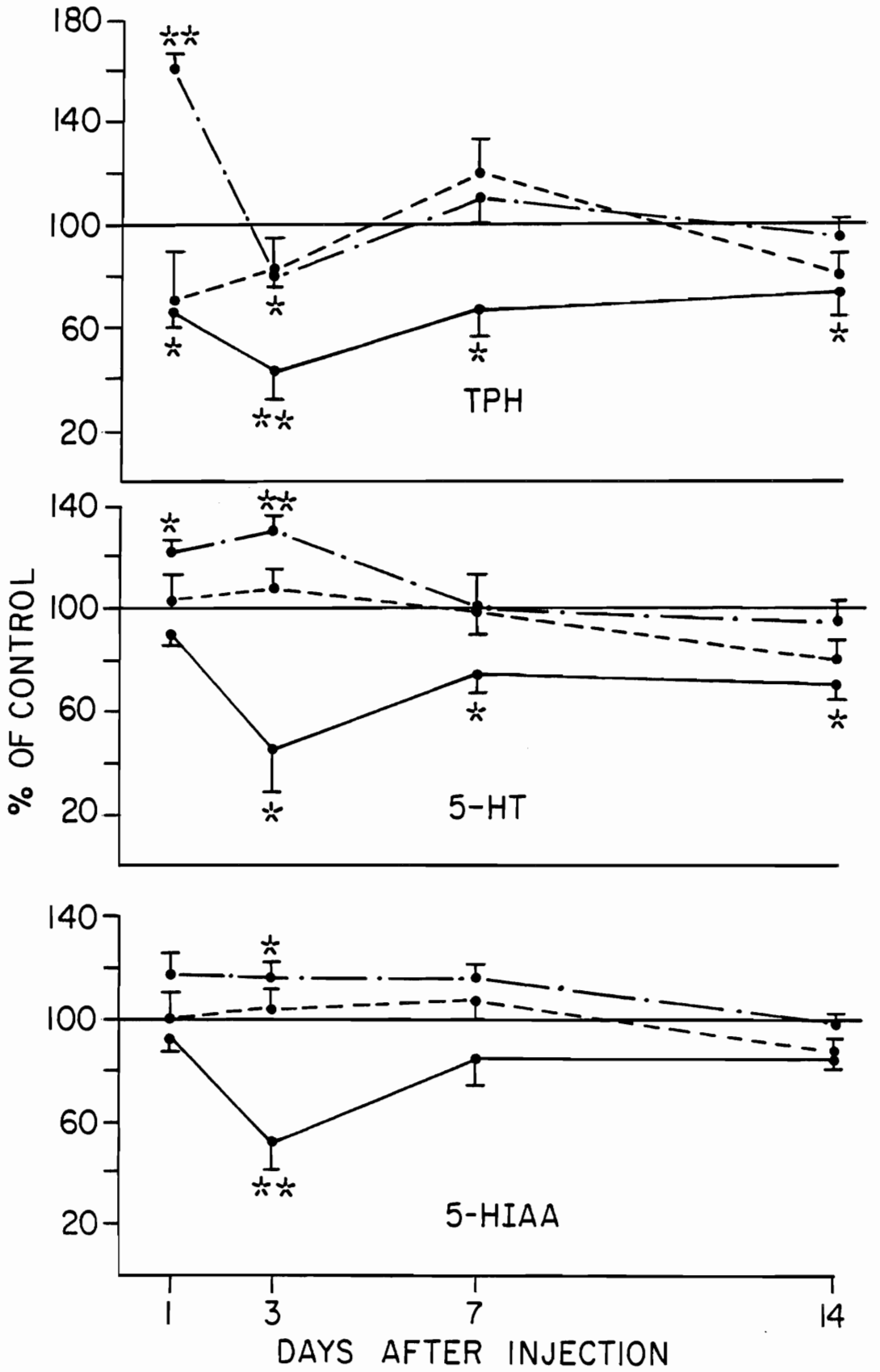

180
140
100
60
20
TPH
140
100
60
20
% OF CONTROL
5-HT
140
100
60
20
5-HIAA
1
3
7
14
DAYS AFTER INJECTION

FIG. 2 Effect of METH and IPR on TPH activity, 5-HT and 5-HIAA concentrations in the hypothalamus at 1, 3, 7 and 14 days after injection. Rats were injected with either saline/METH (– –), IPR/saline (– · –) or IPR/METH (——). IPR (10 mg/kg) or saline were administered 2 hours before injection of METH (17.5 mg/kg) or saline. The number of animals per group were 4 or more. Brackets indicate ± S.E.M. Control values were 107 ± 5.5 nmoles Try oxidized/gm tissue/hr (n = 16), 0.76 ± 0.03 5-HT ng/mg tissue (n = 16) and 0.60 ± 0.04 5-HIAA ng/mg tissue (n = 16).

(*) $p < 0.05$ compared to saline/saline control

(**) $p < 0.005$ compared to saline/saline control

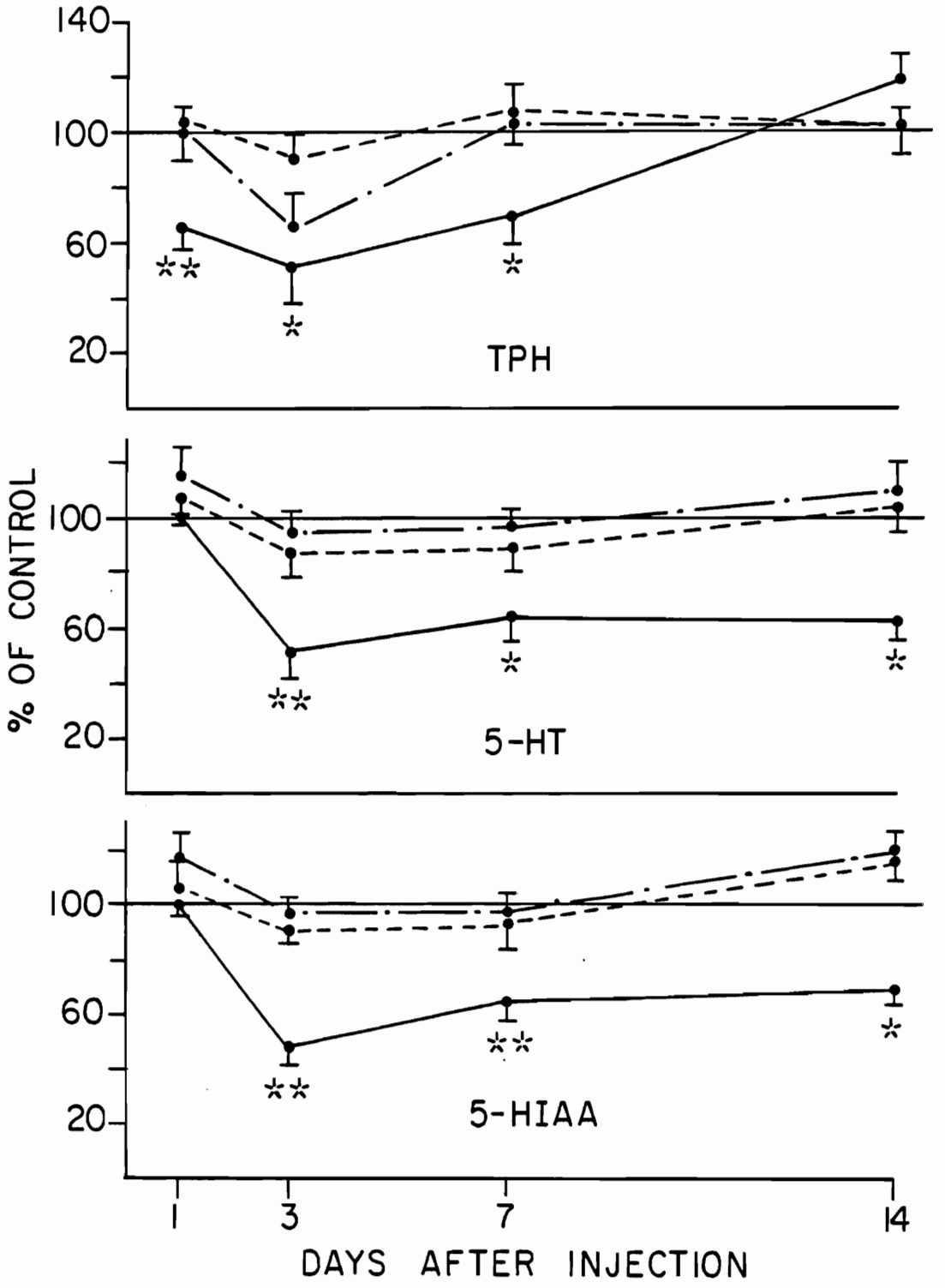

140
100
60
20
TPH
% OF CONTROL
100
60
20
5-HT
100
60
20
5-HIAA
1
3
7
14
DAYS AFTER INJECTION

FIG. 3 Effect of METH and IPR on TPH activity, 5-HT and 5-HIAA concentrations in the neostriatum at 1, 3, 7 and 14 days after injection. Rats were injected with either saline/METH (– –), IPR/saline (– · –) or IPR/METH (——). IPR (10 mg/kg) or saline were administered 2 hours before injection of METH (17.5 mg/kg) or saline. The number of animals per group were 4 or more. Brackets indicate ± S.E.M. Control values were 24.2 ± 1.3 nmoles Try oxidized/gm tissue/hr (n = 16), 0.81 ± 0.04 5-HT ng/mg tissue (n = 16) and 0.60 ± 0.03 5-HIAA ng/mg tissue (n = 16).

(*) $p < 0.05$ compared to saline/saline control

(**) $p < 0.005$ compared to saline/saline control

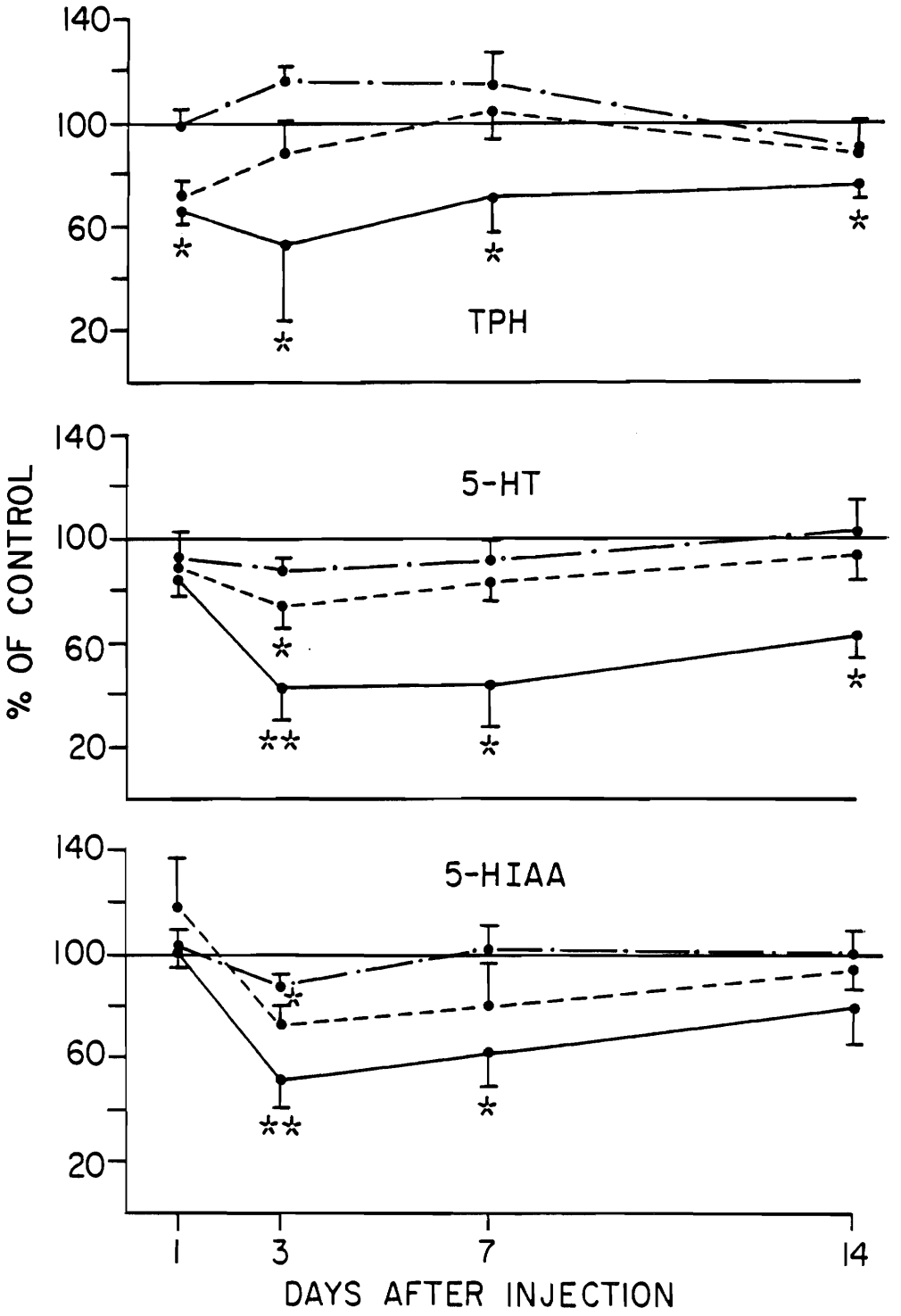
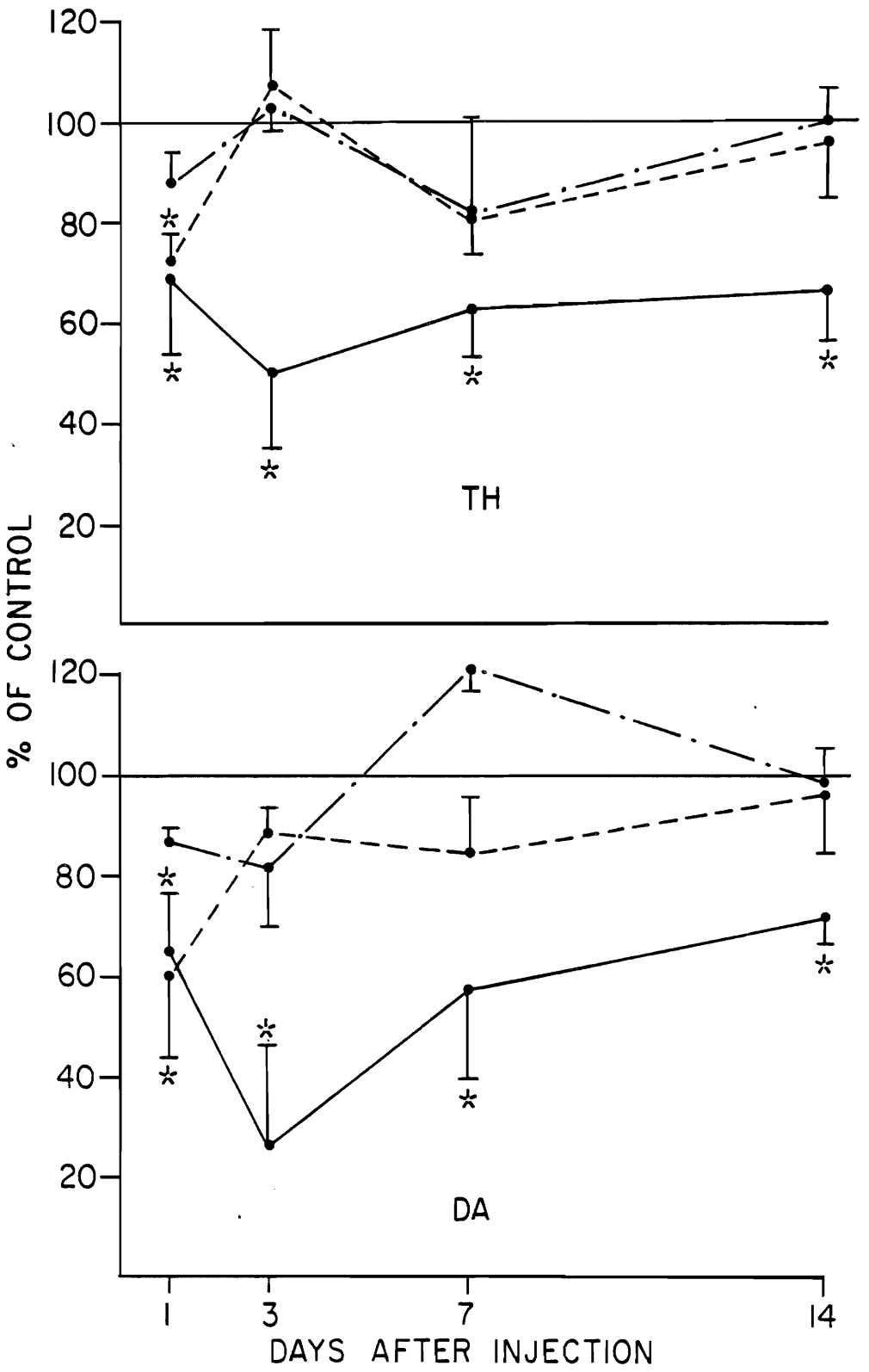

140
100
60
20
TPH
5-HT
5-HIAA
% OF CONTROL
1
3
7
14
DAYS AFTER INJECTION

FIG. 4 Effect of METH and IPR on TH activity and DA concentrations in the neostriatum at 1, 3, 7 and 14 days after injection. Rats were injected with either saline/METH (— —), IPR/saline (— · —) or IPR/METH (——). IPR (10 mg/kg) or saline were administered 2 hours before injection of METH (17.5 mg/kg) or saline. The number of animals per group were 4 or more. Brackets indicate ± S.E.M. Control values were 1550 ± 96 nmoles tyrosine oxidized/gm tissue/hr (n = 16), and 9.4 ± 0.4 DA ng/mg tissue (n = 16).

(*) $p < 0.05$ compared to saline/saline control

(**) $p < 0.005$ compared to saline/saline control


120
100
80
60
40
20
% OF CONTROL
TH
DA
1
3
7
14
DAYS AFTER INJECTION

system, decreases were seen in both TH activity and DA concentrations at 3, 7 and 14 days.

Enzyme activities (TPH and TH), 5-HT, 5-HIAA and DA concentrations were monitored (Figures 1-4) after the injection of iprindole or methamphetamine alone. The latter agent decreased neostriatal TH activity and DA concentrations 1 day after a single injection of 17.5 mg/kg; no changes were observed at 3, 7 or 14 days after injection (Figure 4). TPH activity and indoleamine concentrations, with the exception of neostriatal 5-HT and 5-HIAA concentrations after 3 days (Figure 3), were not significantly different from control values at any time point after a single injection of methamphetamine in the brain regions examined.

Administration of iprindole alone caused changes in cortical TPH activity and indoleamine concentrations 1 and 3 days after injection (Figure 1). Enzyme activity was significantly increased (by 62%) at 1 day and decreased (by 18%) at 3 days; recovery was observed at 7 days. Cortical 5-HT concentrations were increased after 1 day (by 23%); however, in contrast to the enzyme activity, they were still significantly elevated at 3 days (by 31%). 5-HIAA concentrations were only significantly higher than control values at 3 days. Administration of iprindole alone did not cause any significant changes in the serotonergic system of the neostriatum (Figure 3) or hypothalamus (Figure 2) nor in the neostriatal dopaminergic system (Figure 4).

To determine whether lower doses of methamphetamine administered to iprindole-treated rats would produce similar effects in regional serotonergic and neostriatal dopaminergic systems, methamphetamine doses of 2.5, 7.5, 10, 12.5 and 17.5 mg/kg were administered to rats

given 10 mg/kg of iprindole 2 hours previously. The animals were sacrificed at 7 days. Figures 5 to 8 show the effects of increasing doses of methamphetamine on cortical, hypothalamic and neostriatal parameters. Although effects were observed at 10 and 12.5 mg/kg in the cerebral cortex and hypothalamus, significant changes in all 3 brain regions examined were only seen at 17.5 mg/kg.

Freeman and Sulser (1972) had shown that a single dose of iprindole (10 mg/kg) inhibited the para-hydroxylation of amphetamine in the rat. This finding was used by Fuller and Hemrick-Lueke (1980) to show that 7 days after the injection of amphetamine to iprindole-treated rats, cerebral hemisphere and neostriatal DA concentrations were still significantly decreased. The possibility was investigated that a lower dose of this metabolic inhibitor would produce a similar effect. Figures 9 to 12 show the results of experiments using a fixed dose of methamphetamine (17.5 mg/kg) and varying doses of iprindole. Animals were sacrificed 7 days after injection. Doses of iprindole of 5 and 10 mg/kg resulted in decreases in TPH activity, 5-HT and 5-HIAA concentrations in all regions examined (Figures 9-11). In addition, neostriatal TH activity and DA concentrations were decreased (Figure 12). Although a dose of 2 mg/kg also led to a reduction in these parameters, these were only of statistical significance for cortical TPH activity and neostriatal TPH and TH activities.

The effect of iprindole on cortical TPH activity after 24 hours was unexpected. Neckers, Biggio, Moja and Meek (1977) reported that treatments lowering brain Try concentrations increased TPH activity and this appeared to be related to an increase in the Vmax of the enzyme. More specifically, they reported that a single dose of cloimipramine

FIG. 5 Effect of varying doses of METH and a fixed dose of IPR (10 mg/kg) on TPH activity, 5-HT and 5-HIAA concentrations in the cerebral cortex. Animals were sacrificed 7 days after the injection of METH to IPR treated rats. The number of animals per group were 4 or more. Brackets indicate $\pm$ S.E.M. Control values were 28.7 $\pm$ 3.0 nmoles Try oxidized/gm tissue/hr (n = 6), 0.39 $\pm$ 0.03 5-HT ng/mg tissue (n = 5) and 0.26 $\pm$ 0.02 5-HIAA ng/mg tissue (n = 5).

(*) $p < 0.05$ compared to saline control

(**) $p < 0.005$ compared to saline control

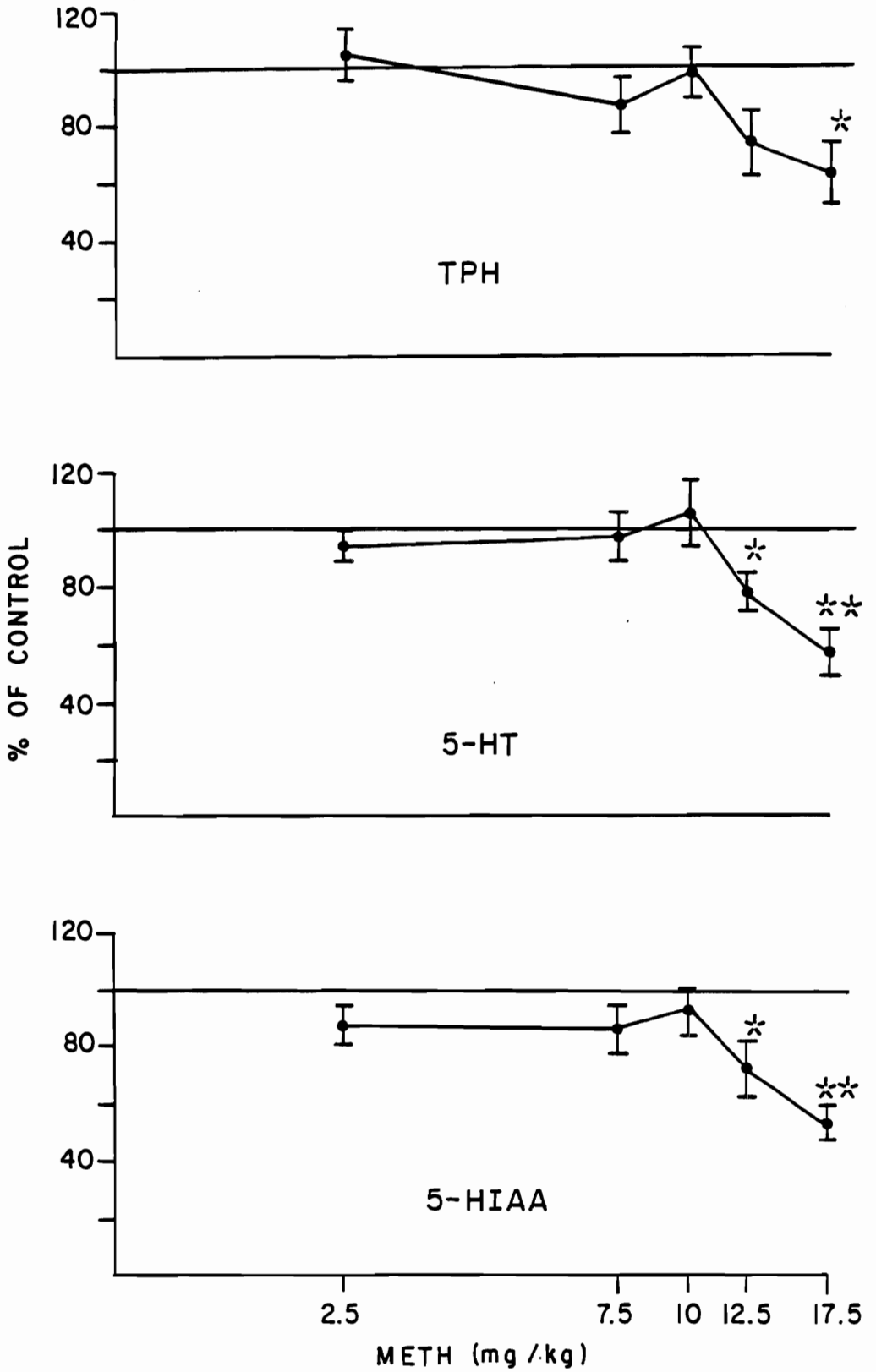

120
80
40
TPH
*
120
80
40
% OF CONTROL
5-HT
*
**
120
80
40
5-HIAA
*
**
2.5
7.5
10
12.5
17.5
METH (mg/kg)

FIG. 6 Effect of varying doses of METH and a fixed dose of IPR (10 mg/kg) on TPH activity, 5-HT and 5-HIAA concentrations in the hypothalamus. Animals were sacrificed 7 days after the injection of METH to IPR treated rats. The number of animals per group were 4 or more. Brackets indicate $\pm$ S.E.M. Control values were 96 $\pm$ 6 nmoles Try oxidized/gm tissue/hr (n = 5), 0.84 $\pm$ 0.06 5-HT ng/mg tissue (n = 5) and 0.63 $\pm$ 0.05 5-HIAA ng/mg tissue (n = 5).

(*) $p < 0.05$ compared to saline control

(**) $p < 0.005$ compared to saline control

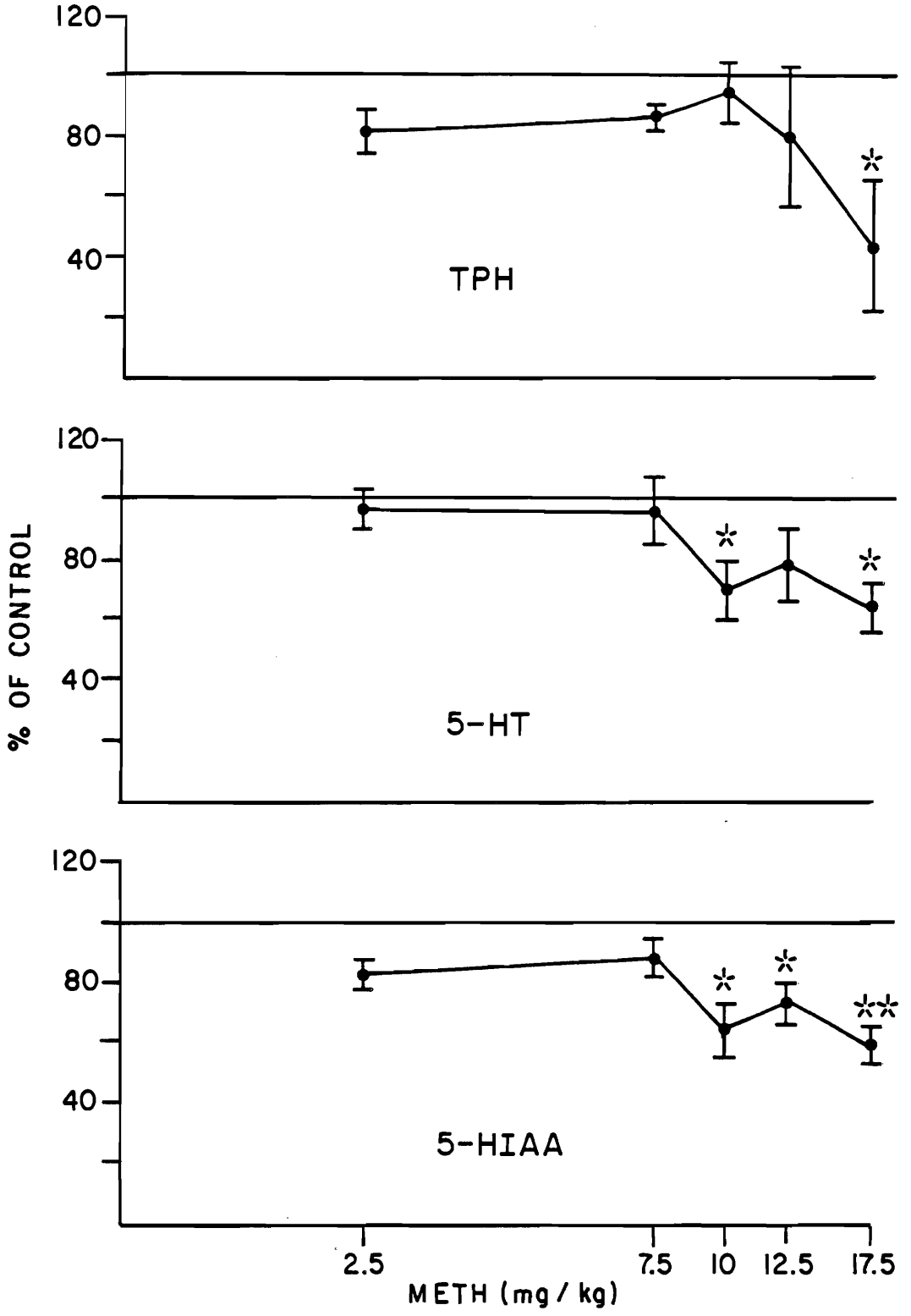

120
80
40
TPH
*
120
80
40
5-HT
*
*
% OF CONTROL
120
80
40
5-HIAA
*
*
**
2.5
7.5
10
12.5
17.5
METH (mg / kg)

FIG. 7 Effect of varying doses of METH and a fixed dose of IPR (10 mg/kg) on TPH activity, 5-HT and 5-HIAA concentrations in the neostriatum. Animals were sacrificed 7 days after the injection of METH to IPR treated rats. The number of animals per group were 4 or more. Brackets indicate $\pm$ S.E.M. Control values were 24.4 $\pm$ 2.8 nmoles Try oxidized/gm tissue/hr (n = 5), 0.86 $\pm$ 0.06 5-HT ng/mg tissue (n = 6) and 0.52 $\pm$ 0.04 5-HIAA ng/mg tissue (n = 6).

(*) $p < 0.05$ compared to saline control

(**) $p < 0.005$ compared to saline control

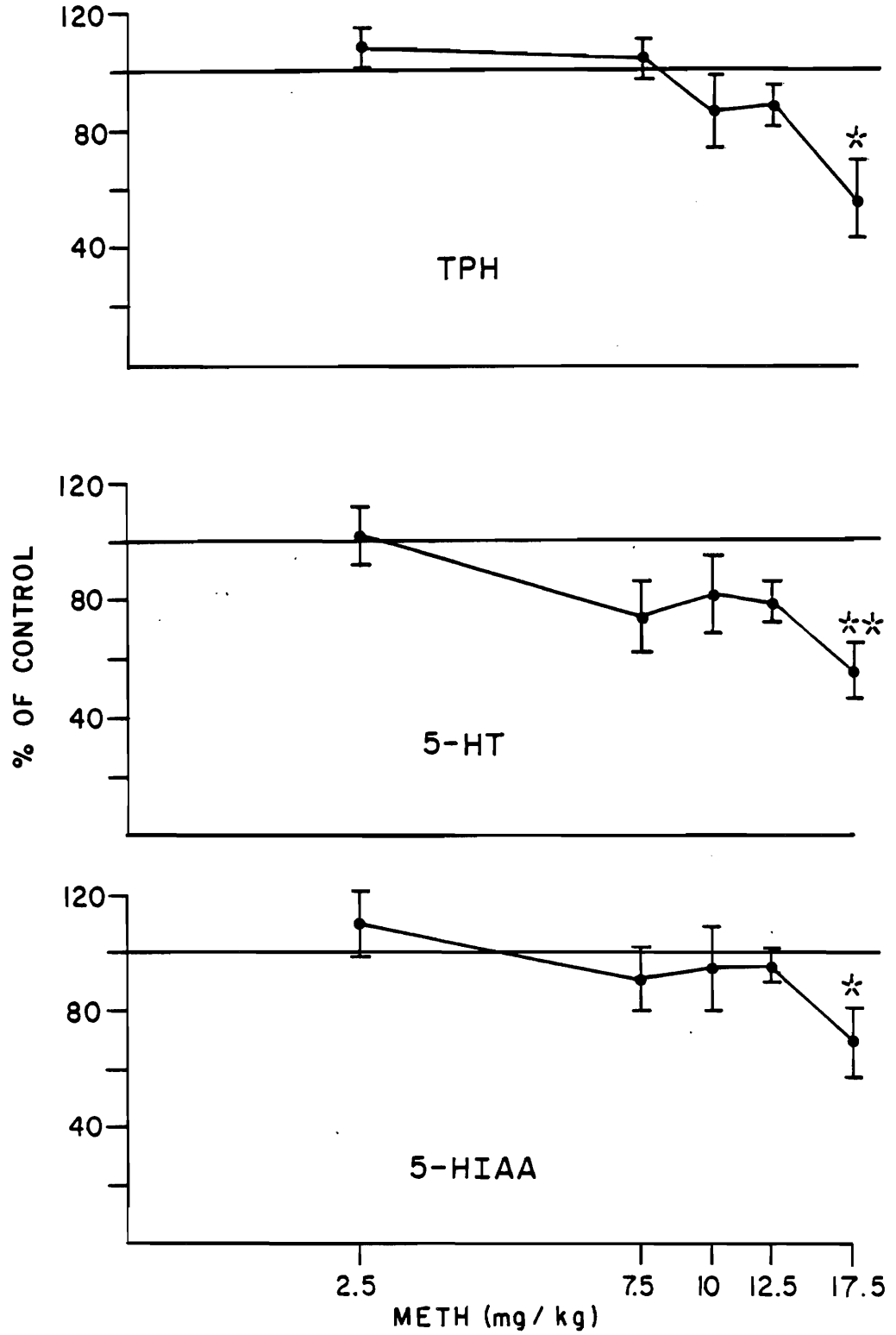

120
80
40
TPH
*
120
80
40
% OF CONTROL
5-HT
**
120
80
40
5-HIAA
*
2.5
7.5
10
12.5
17.5
METH (mg / kg)

FIG. 8 Effect of varying doses of METH and a fixed dose of IPR (10 mg/kg) on TH activity and DA concentrations in the neostriatum. Animals were sacrificed 7 days after the injection of METH to IPR treated rats. The number of animals per group were 4 or more. Brackets indicate $\pm$ S.E.M. Control values were 1985 $\pm$ 158 nmoles tyrosine oxidized/gm tissue/hr (n = 6) and 9.2 $\pm$ 1.0 DA ng/mg tissue (n = 6).

(*) $p < 0.05$ compared to saline control

(**) $p < 0.005$ compared to saline control

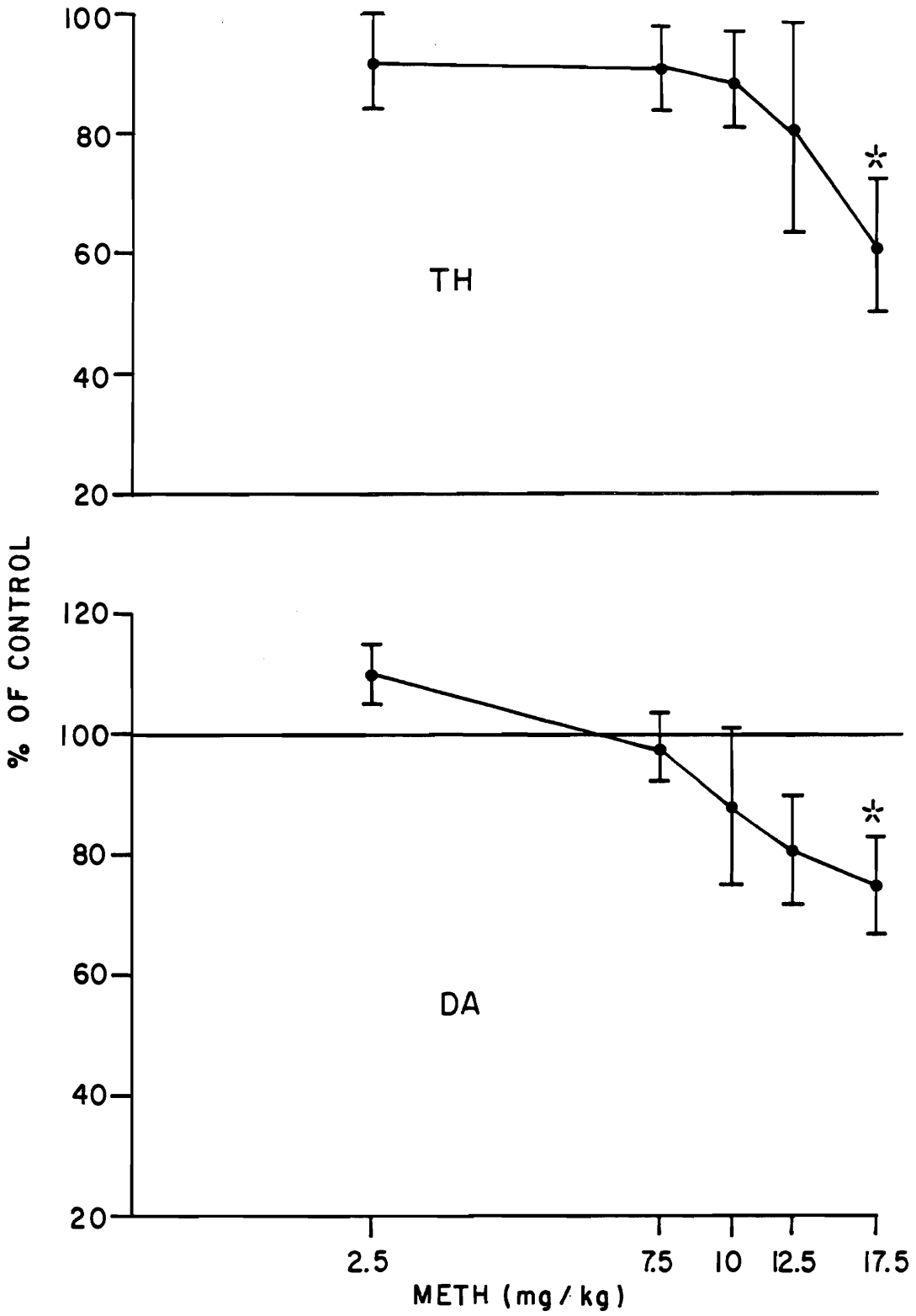

100
80
60
40
20
TH
*
% OF CONTROL
120
100
80
60
40
20
DA
*
2.5
7.5
10
12.5
17.5
METH (mg / kg)

FIG. 9 Effect of varying doses of IPR and a fixed dose of METH (17.5 mg/kg) on TPH activity, 5-HT and 5-HIAA concentrations in the cerebral cortex. Animals were sacrificed 7 days after the injection of METH to IPR treated rats. The number of animals per group were 4 or more. Brackets indicate $\pm$ S.E.M. Control values were 20.8 $\pm$ 0.9 nmoles Try oxidized/gm tissue/hr (n = 5), 0.23 $\pm$ 0.01 5-HT ng/mg tissue (n = 6) and 0.14 $\pm$ 0.01 5-HIAA ng/mg tissue (n = 5).

(*) $p < 0.05$ compared to saline control

(**) $p < 0.005$ compared to saline control

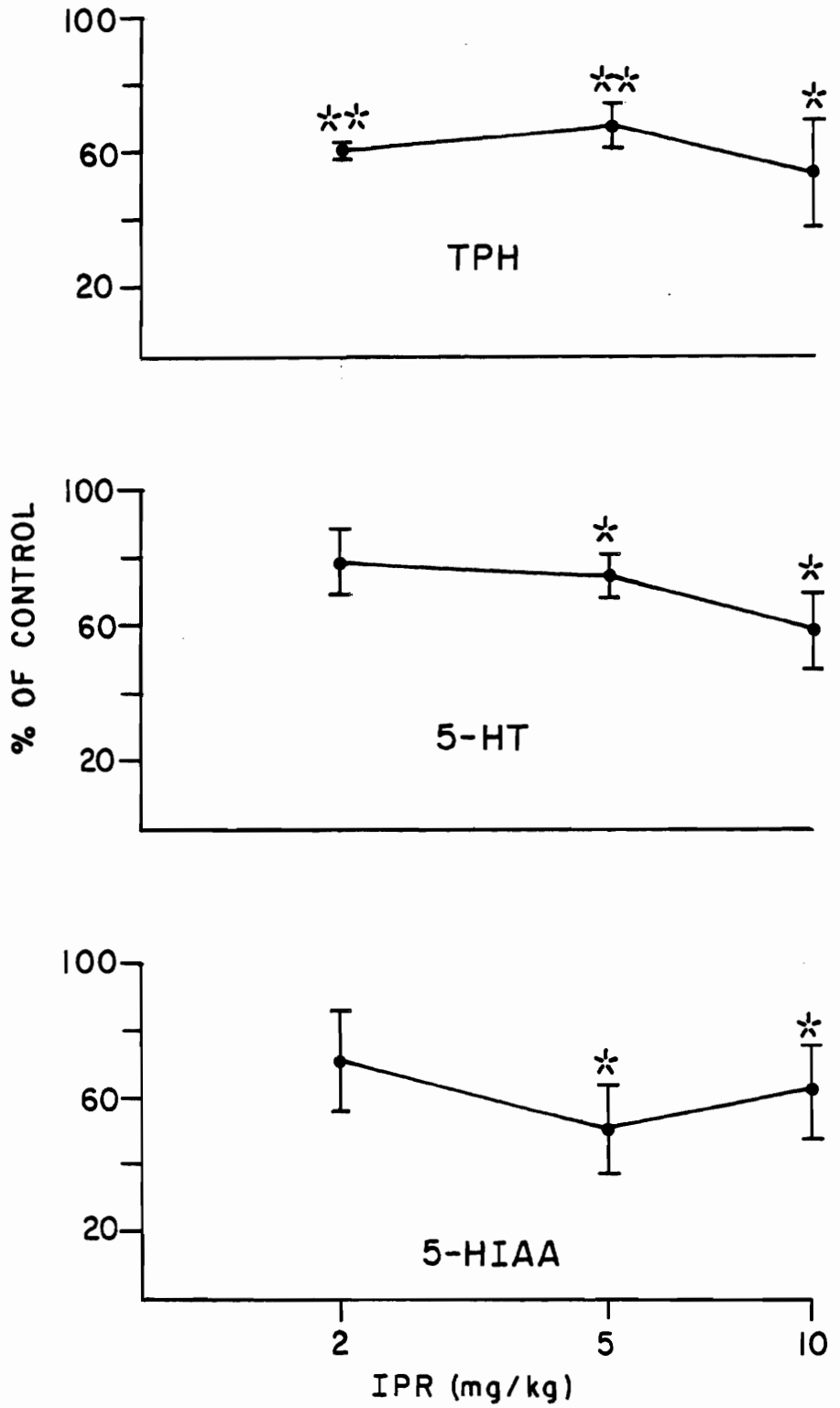

100
60
20
**
**
*
TPH
% OF CONTROL
100
60
20
*
*
5-HT
100
60
20
*
*
5-HIAA
2
5
10
IPR (mg/kg)

FIG. 10 Effect of varying doses of IPR and a fixed dose of METH (17.5 mg/kg) on TPH activity, 5-HT and 5-HIAA concentrations in the hypothalamus. Animals were sacrificed 7 days after the injection of METH to IPR treated rats. The number of animals per group were 4 or more. Brackets indicate $\pm$ S.E.M. Control values for TPH activity were 119 $\pm$ 8 nmoles Try oxidized/gm tissue/hr (n = 6), 1.1 $\pm$ 0.1 5-HT ng/mg tissue (n = 6) and 0.73 $\pm$ 0.04 5-HIAA ng/mg tissue (n = 6).

(*) $p < 0.05$ compared to saline control

(**) $p < 0.005$ compared to saline control

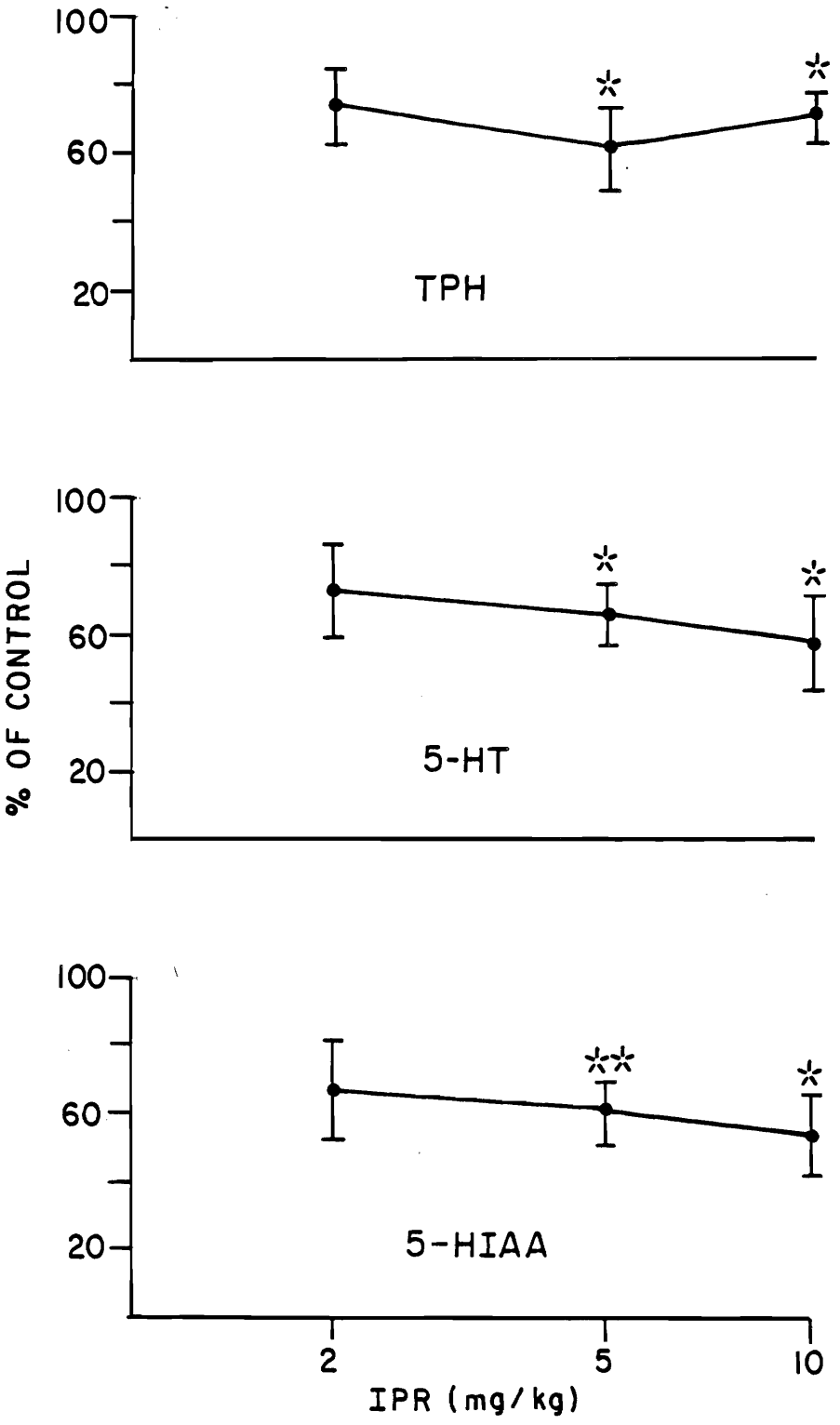

100
60
20
TPH
*
*
100
60
20
% OF CONTROL
5-HT
*
*
100
60
20
5-HIAA
**
*
2
5
10
IPR (mg/kg)

FIG. 11 Effect of varying doses of IPR and a fixed dose of METH (17.5 mg/kg) on TPH activity, 5-HT and 5-HIAA concentrations in the neostriatum. Animals were sacrificed 7 days after the injection of METH to IPR treated rats. The number of animals per group were 4 or more. Brackets indicate $\pm$ S.E.M. Control values were 28.1 $\pm$ 2.2 nmoles Try oxidized/gm tissue/hr (n = 4), 0.53 $\pm$ 0.04 5-HT ng/mg tissue (n = 4) and 0.4 $\pm$ 0.03 5-HIAA ng/mg tissue (n = 4).

(*) $p < 0.05$ compared to saline control

(**) $p < 0.005$ compared to saline control

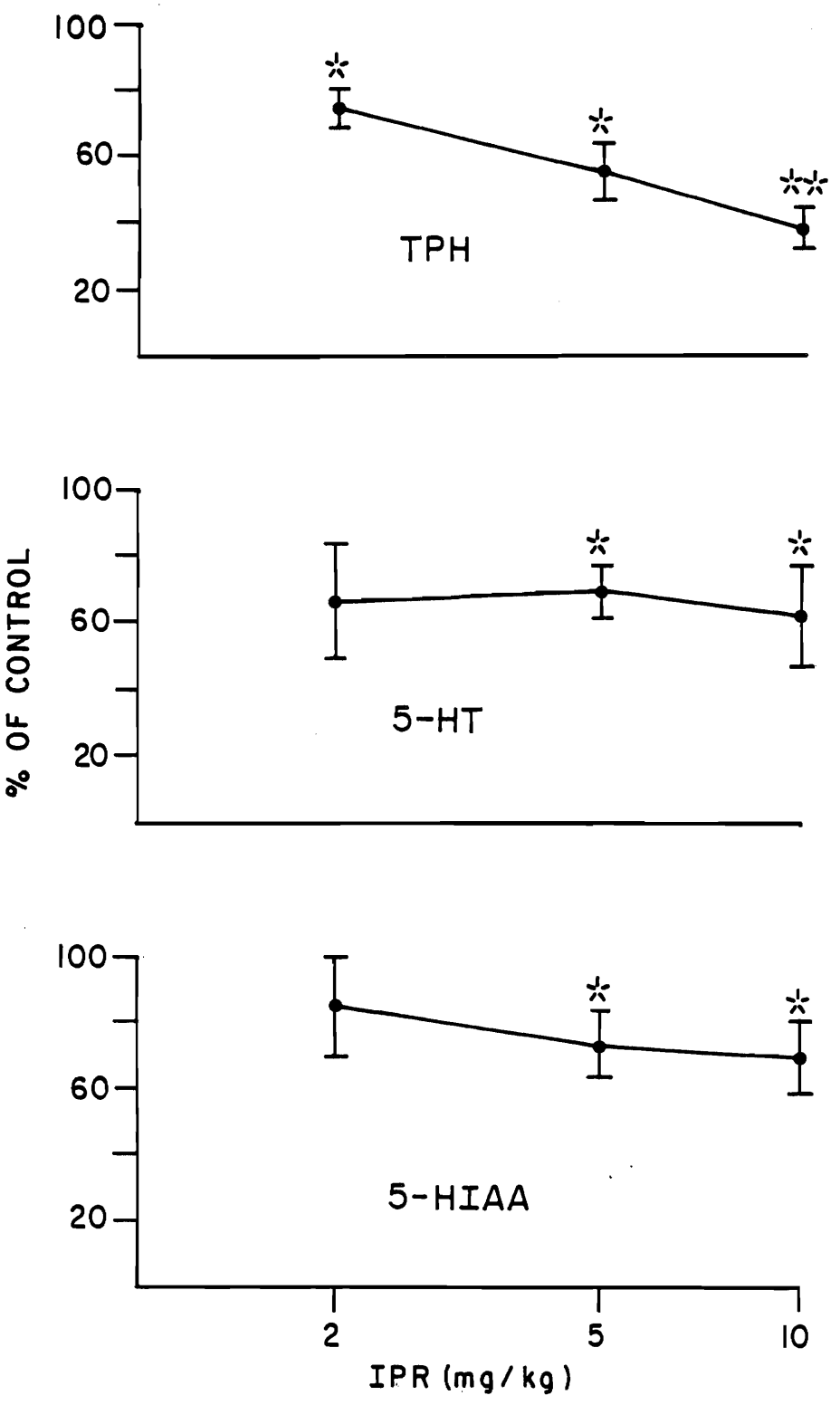
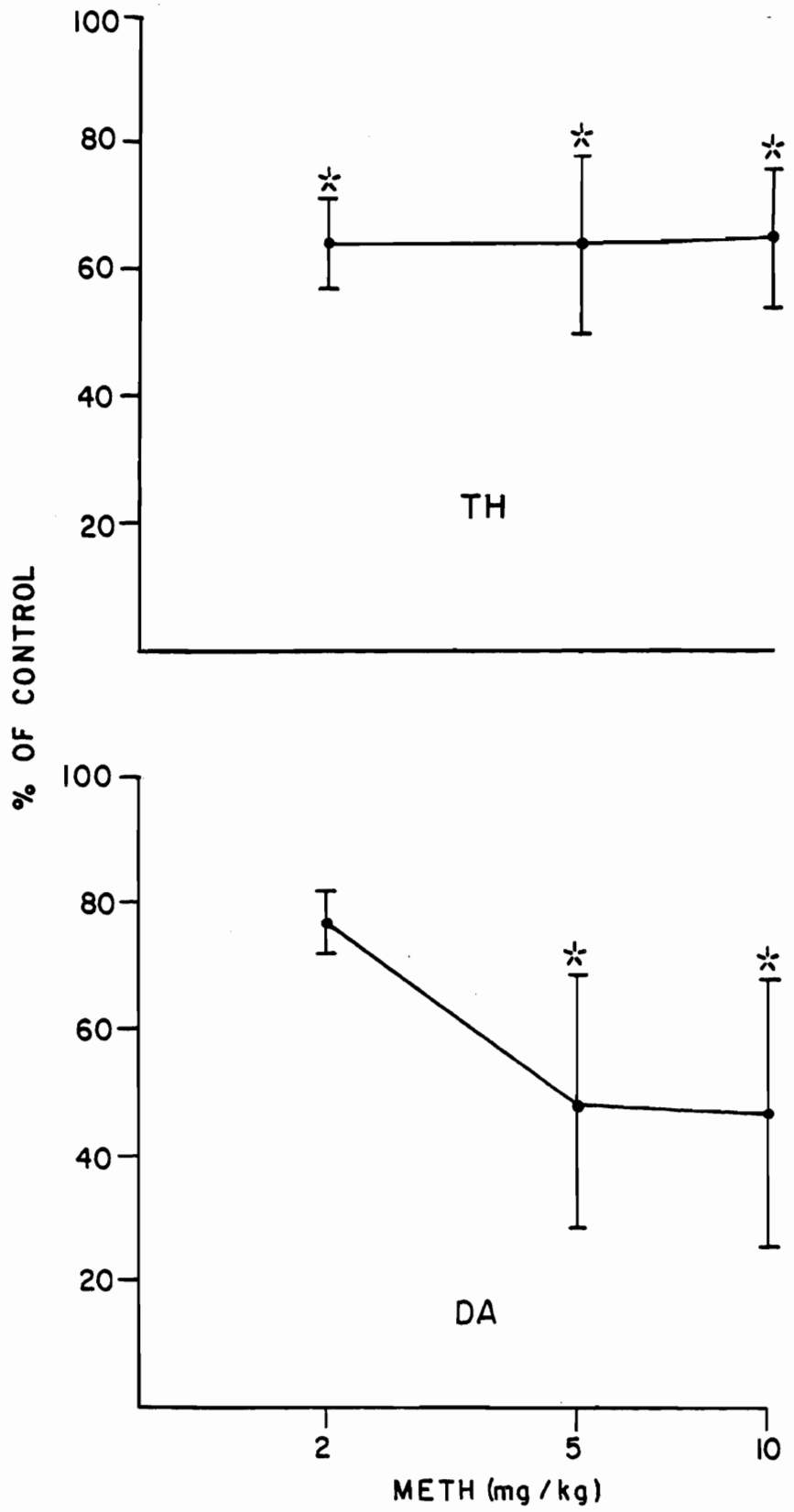

100
60
20
TPH
*
*
**
100
60
20
% OF CONTROL
5-HT
*
*
100
60
20
5-HIAA
*
*
2
5
10
IPR (mg/kg)

FIG. 12 Effect of varying doses of IPR and a fixed dose of METH (17.5 mg/kg) on TH activity and DA concentrations in the neostriatum. Animals were sacrificed 7 days after the injection of METH to IPR treated rats. The number of animals per group were 4 or more. Brackets indicate $\pm$ S.E.M. Control values were 1716 $\pm$ 145 nmoles tyrosine oxidized/mg tissue/hr (n = 4) and 8.9 $\pm$ 0.9 DA ng/mg tissue (n = 4).

(*) $p < 0.05$ compared to saline control

(**) $p < 0.005$ compared to saline control


100
80
60
40
20
TH
100
80
60
40
20
DA
% OF CONTROL
2
5
10
METH (mg / kg)

(15 mg/kg) led to an activation of TPH and a decreased Try content in the rat forebrain as early as 15 minutes after injection. This effect was still present after 4 hours. More recently Badawy and Evans (1981) have shown that rat liver tryptophan pyrrolase is decreased 2 hours after the administration of a single dose (10 mg/kg) of several antidepressants. More particularly, they reported that iprindole increased brain Try concentrations and that desipramine increased brain 5-HT, 5-HIAA and Try. Chlorpromazine was shown to be ineffective.

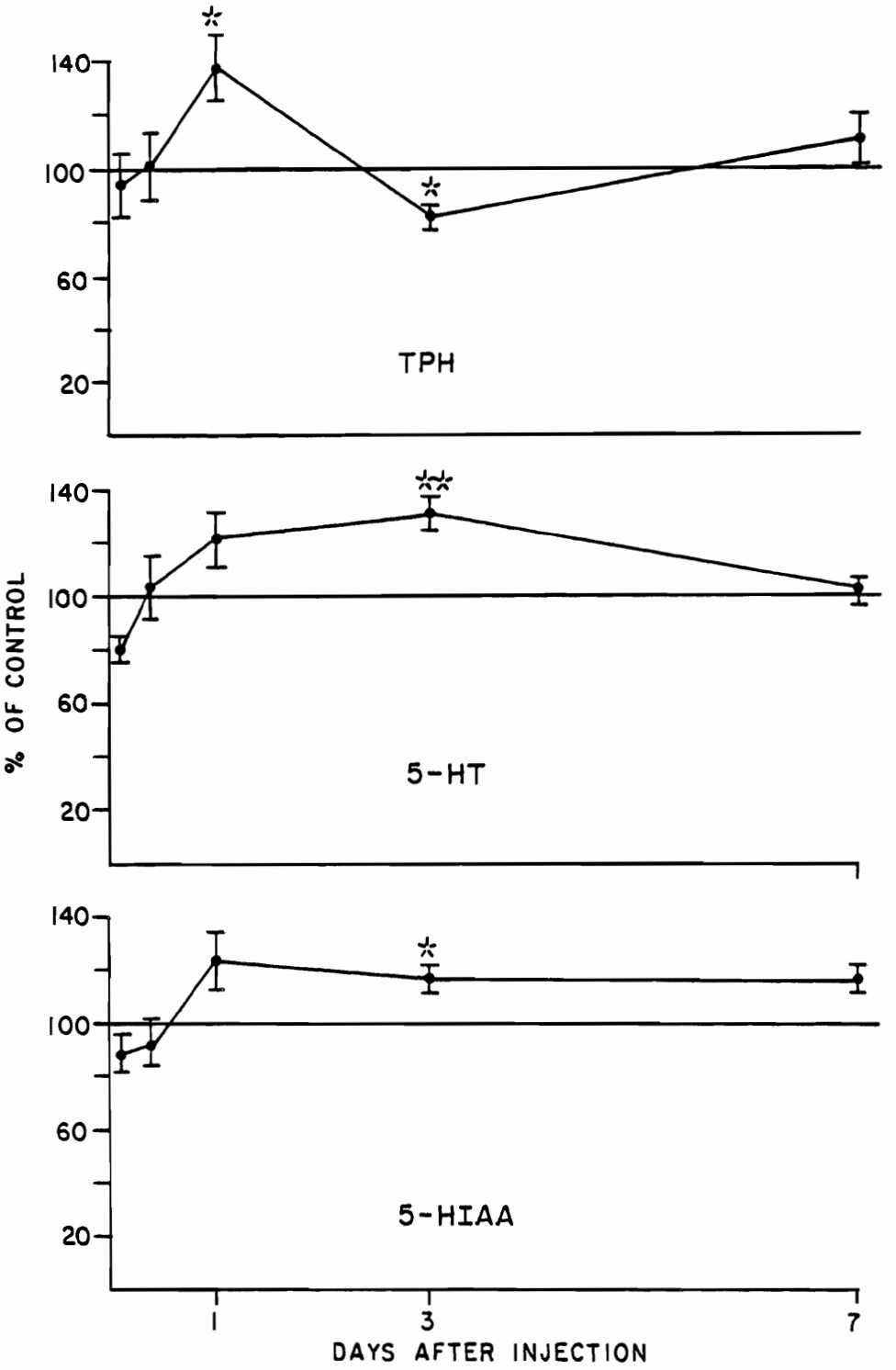
Iprindole, desipramine and cloimipramine are all used clinically as antidepressants. Until recently, it was believed that these drugs acted by inhibiting the reuptake of neurotransmitters (5-HT and NE), thereby increasing their concentration in the synapse. However, this theory has been questioned, in part, because there are a number of therapeutically useful agents which are not efficient uptake blockers. Iprindole is an example of one of these "atypical antidepressants."

To examine further the effects of iprindole on the serotonergic system in the rat brain, experiments were carried out using a single dose of 10 mg/kg. Figure 13 shows the time-course of its effects on cortical TPH activity, 5-HT and 5-HIAA concentrations. TPH activity was increased to 138% of saline control at 1 day and then fell to 82% of control at 3 days. Although there appeared to be an apparent increase in 5-HT and 5-HIAA concentrations at 1 day, this only reached significance at 3 days. After 7 days, all 3 parameters had returned to control values. No significant changes were seen at time points (2 and 6 hours) earlier than 24 hours nor in cortical Try concentrations at any of the time points, except for an increase in Try concentrations at 7 days (saline 4.7 $\pm$ 0.27 ng/mg tissue, iprindole 6.1 $\pm$ 0.23 ng/mg tissue).

FIG. 13 Effect of a single dose of IPR (10 mg/kg) on TPH activity, 5-HT and 5-HIAA concentration at 2 hours, 6 hours, 1 day, 3 days and 7 days in the cerebral cortex. The number of animals per group were 4 or more. Brackets indicate $\pm$ S.E.M. Control values were 24.6 $\pm$ 1.4 nmoles Try oxidized/gm tissue/hr (n = 26), 0.23 $\pm$ 0.02 5-HT ng/mg tissue (n = 28) and 0.18 $\pm$ 0.01 5-HIAA ng/mg tissue (n = 28).

(*) $p < 0.05$ compared to saline control

(**) $p < 0.005$ compared to saline control


140
100
60
20
TPH
5-HT
5-HIAA
% OF CONTROL
1
3
7
DAYS AFTER INJECTION

Tables 3 and 4 show the effects of iprindole 2, 6 and 24 hours after injection on TPH activity, indoleamine and Try concentrations in the hippocampus, hypothalamus and neostriatum and on neostriatal TH activity and DA concentrations. No significant changes in these parameters were observed in any of the brain regions examined. It appears, therefore, from these initial studies that the effects of iprindole on the serotonergic system are limited to the cortex and do not result from changes in cortical Try concentrations.

Table 5 details the effects of several drugs (iprindole, chlorpromazine, amitriptyline, cloimipramine, fluoxetine and desipramine) on cortical TPH activity, indoleamine and Try concentrations. Three drugs, (cloimipramine, amitriptyline and chlorpromazine) all increased TPH activity and 5-HT concentrations, in a similar manner to iprindole. Cloimipramine and chlorpromazine also increased 5-HIAA concentrations. Although desipramine did not increase TPH activity, 5-HT and 5-HIAA concentrations were significantly elevated. The only agent to affect Try concentration was cloimipramine, which increased it significantly by 29%; this is in contrast to the results reported by Neckers et al. (1977) and Badawy and Evans (1981), although neither group monitored the amino acid concentrations at 24 hours.

Effects of Phencyclidine

The primary aim of the study with PCP was to compare its effects on the neostriatal serotonergic system of the rat brain with methamphetamine-induced changes. Acute and chronic injection of methamphetamine have been shown to decrease neostriatal TPH activity and 5-HT concentrations. After an acute injection of methamphetamine

TABLE 3

TPH ACTIVITY, 5-HT, 5-HIAA AND Try CONCENTRATIONS IN THE HIPPOCAMPUS, HYPOTHALAMUS AND NEOSTRIATUM AFTER A SINGLE INJECTION OF IPR (10 mg/kg)

(Expressed as % of Control)

	Hours					
	2		6		24	
	n	$\bar{x} \pm$ S.E.M.	n	$\bar{x} \pm$ S.E.M.	n	$\bar{x} \pm$ S.E.M.
Hippocampus						
TPH	6	97 ± 9.4	6	106 ± 8.9	5	92 ± 4.3
5-HT	6	95 ± 5.2	6	93 ± 6.2	6	115 ± 4.8
5-HIAA	6	89 ± 5.6	6	100 ± 4.5	6	106 ± 3.2
Try	6	98 ± 6.2	6	94 ± 3.6	6	103 ± 2.6
Hypothalamus						
TPH	6	92 ± 4.5	6	100 ± 7.7	6	101 ± 11
5-HT	5	98 ± 2.3	5	68 ± 14	6	85 ± 5
5-HIAA	5	101 ± 4.3	5	107 ± 15	6	87 ± 5.9
Try	5	105 ± 7.8	5	93 ± 10	5	96 ± 6.2
Neostriatum						
TPH	4	88 ± 3.5	5	111 ± 9.4	6	82 ± 17
5-HT	5	93 ± 5.2	6	107 ± 9.3	6	104 ± 7.0
5-HIAA	6	92 ± 6.2	6	115 ± 7.6	6	113 ± 6.1
Try	6	97 ± 7.1	6	102 ± 11	6	124 ± 4.2

Control values were as follows: hippocampus; 31.9 ± 1.3 nmoles Try oxidized/gm tissue/hr (n = 16), 0.40 ± 0.014 5-HT ng/mg tissue (n = 17), 0.45 ± 0.013 5-HIAA ng/mg tissue (n = 17) and 4.8 ± 0.5 Try ng/mg tissue (n = 17); hypothalamus, 69.2 ± 4.3 nmoles Try oxidized/gm tissue/hr (n = 16), 0.63 ± 0.05 5-HT ng/mg tissue (n = 17), 0.31 ± 0.015 5-HIAA ng/mg tissue (n = 17) and 3.6 ± 0.7 Try ng/mg tissue (n = 17); neostriatum, 27.8 ± 2.6 nmoles Try oxidized/gm tissue/hr (n = 16), 0.40 ± 0.039 5-HT ng/mg tissue (n = 16), 0.35 ± 0.02 5-HIAA ng/mg tissue (n = 16) and 3.42 ± 0.27 Try ng/mg tissue (n = 16).

TABLE 4

TH ACTIVITY AND DA CONCENTRATIONS IN THE NEOSTRIATUM

AFTER A SINGLE INJECTION OF IPR (10 mg/kg)

(Expressed as % of Control)

	Hours					
	2		6		24	
	n	$\bar{x} \pm$ S.E.M.	n	$\bar{x} \pm$ S.E.M.	n	$\bar{x} \pm$ S.E.M.
TH	6	81 $\pm$ 9.0	6	130 $\pm$ 5.4	6	108 $\pm$ 4.0
DA	6	108 $\pm$ 5.2	6	104 $\pm$ 4.5	6	105 $\pm$ 12

Control values were 1216 $\pm$ 69 nmoles tyrosine oxidized/gm tissue/hr (n = 16) and 9.5 $\pm$ 0.38 DA ng/mg tissue (n = 18).

TABLE 5

CORTICAL TPH ACTIVITY, 5-HT, 5-HIAA AND Try CONCENTRATIONS TWENTY FOUR HOURS AFTER A SINGLE DOSE (10 mg/kg) OF SEVERAL ANTIDEPRESSANTS

(Expressed as % of Control)

	TPH		5-HT		5-HIAA		Try	
	n	$\bar{x} \pm$ S.E.M.	n	$\bar{x} \pm$ S.E.M.	n	$\bar{x} \pm$ S.E.M.	n	$\bar{x} \pm$ S.E.M.
Saline	6	100 ± 7.2	6	100 ± 6.3	6	100 ± 9.8	5	100 ± 6.7
Iprindole	6	135 ± 5.1**	7	119 ± 5.3*	7	119 ± 5.8	7	111 ± 5.7
Cloimipramine	6	139 ± 3.9**	7	159 ± 11.1**	7	140 ± 7.7*	7	129 ± 5.2*
Amitriptyline	7	137 ± 8.1**	6	120 ± 4.7*	6	125 ± 8.6	6	104 ± 3.9
Chlorpromazine	7	144 ± 3.2**	7	144 ± 6.6**	7	137 ± 5.7*	7	106 ± 6.0
Fluoxetine	7	100 ± 8.5	6	128 ± 5.9*	6	88 ± 7.8	6	106 ± 7.4
Desipramine	7	111 ± 7.2	7	137 ± 7.9**	7	132 ± 7.3*	7	117 ± 5.4

Control values were 21.1 ± 1.5 nmoles Try oxidized/gm tissue/hr (n = 6), 0.19 ± 0.009 5-HT ng/mg tissue (n = 6), 0.13 ± 0.006 5-HIAA ng/mg (n = 6) and 2.6 ± 0.13 Try ng/mg (n = 6).

(*) $p < 0.05$ compared to saline control

(**) $p < 0.005$ compared to saline control

(10 mg/kg) a decrease in enzyme activity is observed after 15 minutes; however, 5-HT concentrations are not significantly lowered until 30 minutes. Subacute administration of methamphetamine has also been shown to decrease neostriatal TPH activity, 5-HT and 5-HIAA concentrations (Bakhit et al., in press).

Initial experiments, involving the subacute administration of PCP (see Methods) showed no statistical difference between neostriatal indoleamine concentrations of treated and saline control animals (Table 6). Smith et al. (1980), however, had reported that neostriatal TH activity was decreased 15 minutes after an acute injection of PCP (10 mg/kg) and 15 minutes and 24 hours after 30 daily injections. For this reason, neostriatal 5-HT and 5-HIAA concentrations were monitored after both chronic and acute administration of the drug. Table 7 shows the results of the chronic study; no statistical difference was observed in either parameter. The effects of an acute injection of PCP on neostriatal indoleamine concentrations are shown in Figure 14. When compared to their own saline control, 5-HIAA concentrations were significantly elevated at 15 and 30 minutes, but not at any other time point examined (10, 20, 60 and 120 minutes). 5-HT concentrations were not significantly different from saline controls at any time point examined. It is interesting to note that there appears to be a cyclical variation in both 5-HT and 5-HIAA concentrations between 15 and 30 minutes. It is clear, from these limited studies, that although PCP may have a subtle effect on the neostriatal serotonergic system, it does not cause the same dramatic effects as methamphetamine administration.

The effects of PCP on glutamic acid decarboxylase (GAD) activity were also examined. Acute and chronic injections of the drug produced

FIG. 14 Time-course of the effects of a single injection (10 mg/kg) of phencyclidine (PCP) on 5-HT (– –) and 5-HIAA (——) concentrations in the neostriatum. The number of animals per group were 5 or more. Brackets indicate ± S.E.M. Control values were 1.08 ± 0.08 5-HT ng/mg tissue (n = 30) and 0.85 ± 0.06 5-HIAA ng/mg tissue (n = 30).

(*) $p < 0.05$ compared to saline control

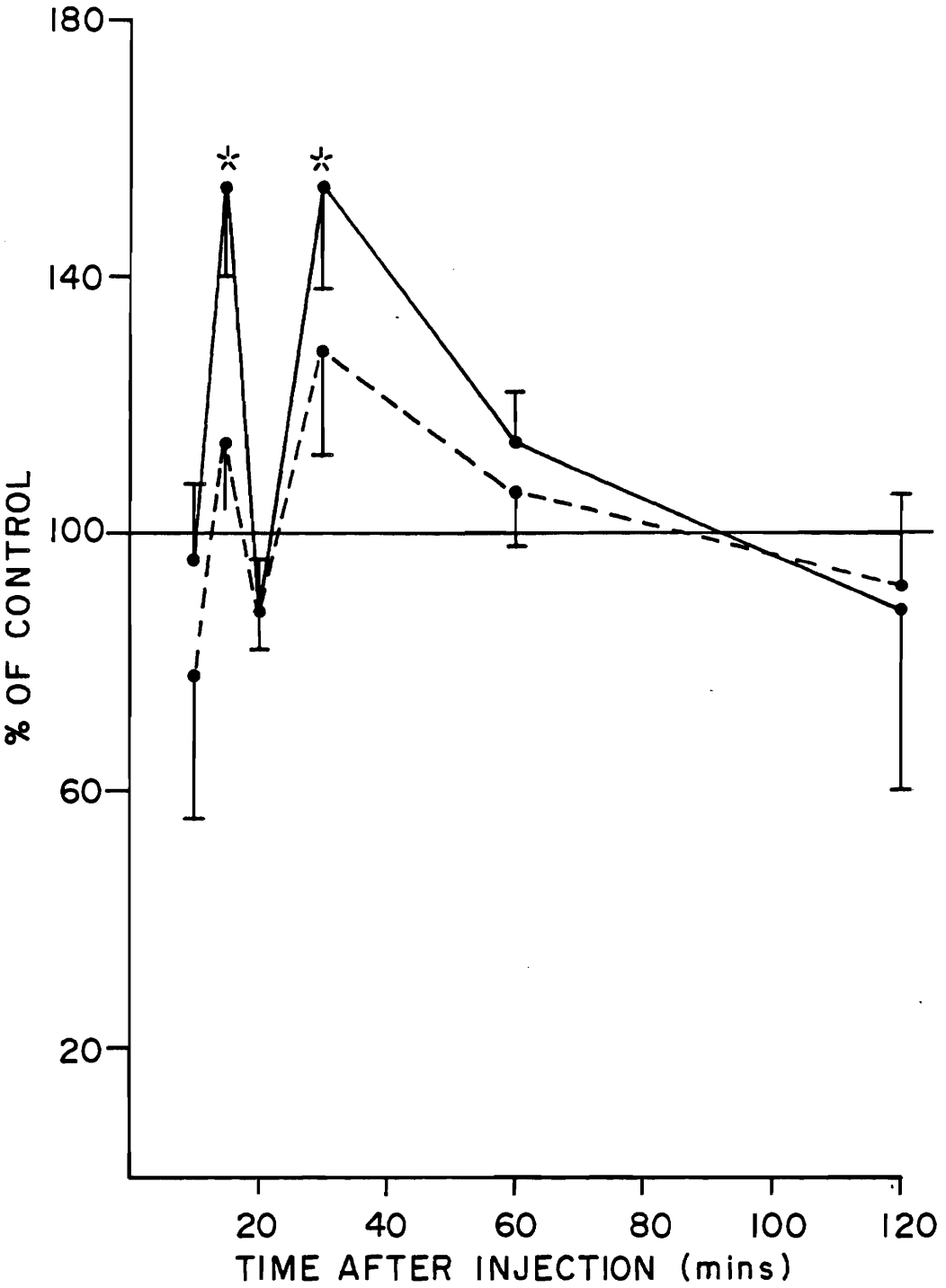

180
140
100
60
20
% OF CONTROL
*
*
20
40
60
80
100
120
TIME AFTER INJECTION (mins)

TABLE 6

EFFECTS OF SUBACUTE PCP TREATMENT ON 5-HT AND 5-HIAA CONCENTRATIONS (ng/mg tissue) IN THE NEOSTRIATUM

	5-HT		5-HIAA	
	n	$\bar{x} \pm$ S.E.M.	n	$\bar{x} \pm$ S.E.M.
Saline	4	0.89 ± 0.06	4	0.58 ± 0.1
2.5 mg/kg	4	0.81 ± 0.20	4	0.50 ± 0.13
5 mg/kg	4	0.95 ± 0.15	4	0.66 ± 0.1
10 mg/kg	4	1.10 ± 0.23	4	0.89 ± 0.21

Animals were sacrificed 12 hours after 4 injections of either PCP or saline.

TABLE 7

EFFECTS OF CHRONIC PCP TREATMENT ON 5-HT AND 5-HIAA CONCENTRATIONS (ng/mg tissue) IN THE NEOSTRIATUM

	5-HT				5-HIAA			
	15 Min.		24 Hrs.		15 Min.		24 Hrs.	
	n	$\bar{x} \pm$ S.E.M.	n	$\bar{x} \pm$ S.E.M.	n	$\bar{x} \pm$ S.E.M.	n	$\bar{x} \pm$ S.E.M.
Saline	5	0.72 ± 0.07	4	0.76 ± 0.08	5	0.42 ± 0.035	4	0.52 ± 0.06
PCP	9	0.79 ± 0.03	9	0.87 ± 0.04	9	0.44 ± 0.015	9	0.48 ± 0.02

Animals were sacrificed 15 minutes and 24 hours after 30 daily injections of either PCP (10 mg/kg) or saline.

no significant changes in enzyme activity in any of the regions examined (Table 8); however, subacute administration of 10 mg/kg every 3 hours for 12 hours resulted in a 16% decrease of cerebellar GAD activity 12 hours after the last injection. No changes were seen in hippocampal, cortical or neostriatal enzyme activity (Table 9). Figure 15 shows a dose-response curve for the decrease in cerebellar enzyme activity. Although doses of 1 and 2.5 mg/kg produced no statistically significant decrease, enzyme activity was depressed after doses of 5 and 10 mg/kg. The time-course of this depression was monitored by sacrificing animals at various times after the subacute administration of 10 mg/kg of PCP. Figure 16 details the results of this experiment. GAD activity was only significantly decreased 6 and 12 hours after the last injection.

FIG. 15 Dose response for the effects of subacute administration of PCP on GAD activity in the cerebellum. Animals were sacrificed 12 hours after the last injection. The number of animals per group were 9 or more. Brackets indicate $\pm$ S.E.M. Control value was 4717 $\pm$ 235 nmoles glutamate oxidized/gm tissue/hr (n = 9).

(*) $p < 0.05$ compared to saline control

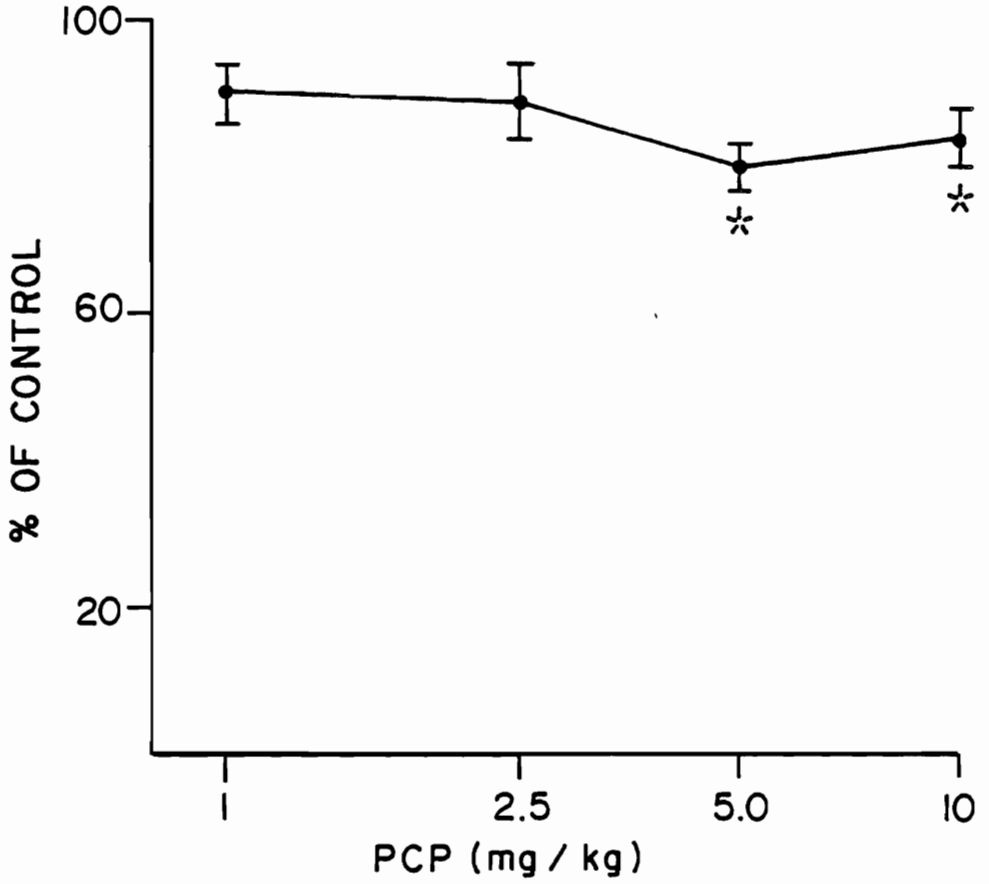

100
60
20
% OF CONTROL
*
*
1
2.5
5.0
10
PCP (mg / kg)

FIG. 16 Time-course of the effects of subacute administration of PCP (10 mg/kg) on GAD activity in the cerebellum. The number of animals per group were 4 or more. Brackets indicate $\pm$ S.E.M. Control value was 3498 $\pm$ 71 nmoles glutamate oxidized/gm tissue/hr (n = 22).

(*) $p < 0.05$ compared to saline control

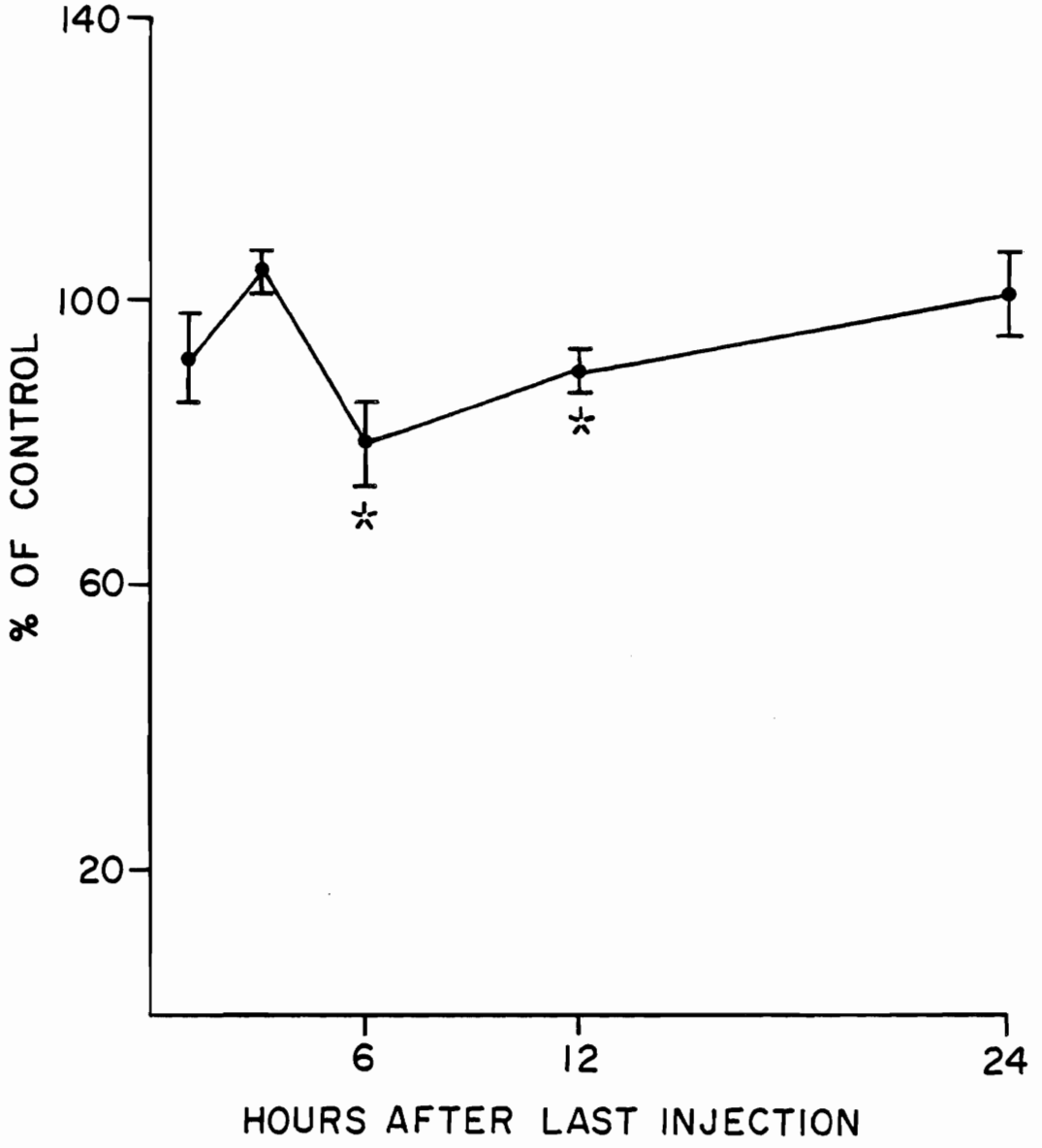

140
100
60
20
% OF CONTROL
*
*
6
12
24
HOURS AFTER LAST INJECTION

TABLE 8

EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF PCP ON GAD ACTIVITY IN THE CEREBELLUM AND CEREBRAL CORTEX

(Expressed as % of Control)

	Cerebellum		Cerebral Cortex	
	n	$\bar{x} \pm$ S.E.M.	n	$\bar{x} \pm$ S.E.M.
Acute:				
10 Mins	4	104 ± 4.3	6	113 ± 5.9
15 Mins	6	98 ± 5.1	4	118 ± 8.1
20 Mins	6	120 ± 6.8	4	108 ± 4.1
30 Mins	5	96 ± 2.0	4	110 ± 6.9
60 Mins	5	108 ± 3.5	6	96 ± 6.3
120 Mins	5	110 ± 0.9*	6	91 ± 3.2
Chronic:				
15 Mins	10	109 ± 11	10	101 ± 4.7
24 Hrs	9	80 ± 19	9	96 ± 3.2

For the acute group, animals were administered 10 mg/kg of PCP or saline and sacrificed at the time points shown.

For the chronic group, animals were sacrificed 15 minutes and 24 hours after 30 daily injections of PCP (10 mg/kg) or saline.

Control GAD activities (nmoles glutamate oxidized/gm tissue/hour) were as follows: Cerebellum 3399 ± 266 (n = 40) and cerebral cortex 8342 ± 341 (n = 40).

(*) $p < 0.05$ compared to saline control

TABLE 9

EFFECTS OF SUBACUTE PCP TREATMENT ON GAD ACTIVITY (nmoles glutamate oxidized/gm tissue/hr) IN THE HIPPOCAMPUS, CEREBRAL CORTEX AND NEOSTRIATUM

	Hippocampus		Cerebral Cortex		Neostriatum	
	n	$\bar{x} \pm$ S.E.M.	n	$\bar{x} \pm$ S.E.M.	n	$\bar{x} \pm$ S.E.M.
Saline	9	8372 ± 459	9	8995 ± 225	7	9705 ± 1020
1 mg/kg	10	9306 ± 904	10	8508 ± 187	10	9508 ± 492
2.5 mg/kg	10	7977 ± 460	10	8573 ± 263	10	10680 ± 652
10 mg/kg	9	7612 ± 225	10	8489 ± 353	10	9025 ± 705

Animals were sacrificed 12 hours after 4 injections of either saline or PCP.

DISCUSSION

In the present study, the effects of a combined dose of iprindole and methamphetamine on the serotonergic system in the neostriatum, cerebral cortex and hypothalamus and on the dopaminergic system in the neostriatum were examined. Freeman and Sulser (1972) have previously shown that iprindole inhibited the para-hydroxylation of amphetamine in the rat, increasing the brain half-life of amphetamine 4 to 5 fold. Inhibition of methamphetamine metabolism by iprindole would therefore lead to a prolongation of its activity. The results of combined administration show that 14 days after administration, cortical (Figure 1) and neostriatal (Figure 3) TPH activity were still significantly decreased. These decreases in enzyme activity correlated well with decreases in indoleamine concentrations. In contrast, hypothalamic indoleamine concentrations were still depressed after 14 days but TPH activity had returned to control values (Figure 2). Previous work (Bakhit et al., in press) has shown that the hypothalamic serotonergic system is relatively resistant to the effects of methamphetamine; recovery in enzyme activity and indoleamine concentrations was observed 110 days after the subacute administration of methamphetamine. Ricaurte et al. (1980) also showed that methamphetamine treatment decreased the concentration of 5-HT in several brain regions and that the same treatment reduced the number of 5-HT uptake sites. It is possible that recovery in hypothalamic enzyme activity occurs before recovery in the number of 5-HT uptake sites, leading to a continued decrease of indoleamine concentrations.

Three and 7 days after the administration of methamphetamine to iprindole-treated rats, TPH activity and 5-HT concentrations were decreased in all 3 brain regions examined (Figures 1-3). The most pronounced depression in these parameters was seen at 3 days, with a partial recovery being observed after 7 days. After 1 day, TPH activity in the cerebral cortex, neostriatum and hypothalamus was decreased by more than 30%, whereas indoleamine concentrations were not significantly different from animals administered saline. These findings suggest that a primary action of methamphetamine on the serotonergic system is to cause the inhibition of TPH, the rate-limiting enzyme in the synthesis of 5-HT. Similar results were obtained using an acute injection of methamphetamine; neostriatal, hypothalamic and cortical TPH activities were decreased before 5-HT concentrations.

Although methamphetamine appears to have a primary action on TPH, the mechanism by which it depresses the enzyme is not yet apparent. Knapp et al. (1974) suggested that it acts directly on TPH; however, they did not find an effect of amphetamine on the enzyme in vitro or on synaptosomes. They also ruled out the possibility that the drug was acting through a metabolic product, an observation supported by the present study using a metabolic inhibitor. The possibility exists that amphetamine (and methamphetamine) affect TPH and TH activity by altering the concentration of the cosubstrate (reduced pterin tetrahydrobiopterin) for both enzymes (Mandell, Bullard, Yellin and Russo, 1980).

The neurotoxic effects of methamphetamine on the serotonergic system reported in this study are similar to those seen after injection of halogenated amphetamines. p-Chloroamphetamine decreased 5-HT concentrations and TPH activity in several brian regions (Sanders-Bush,

et al., 1975) with the neostriatum and cerebral cortex being particularly sensitive. Increasing the duration of action of methamphetamine by inhibiting its metabolism results in prolonged effects on the serotonergic system of the cerebral cortex and neostriatum (Figures 1 and 3). The hypothalamus appears to be relatively resistant to the effects of methamphetamine and p-chloroamphetamine. Although these effects of administering methamphetamine to iprindole-treated rats may not be as long-lasting as those of p-chloroamphetamine treatment, it is interesting to note that fenfluramine, another halogenated amphetamine, did not produce as prolonged a depression of enzyme activity and 5-HT concentrations as p-chloroamphetamine. In further support of the hypothesis that methamphetamine and the halogenated amphetamines may produce a similar neurotoxic effect on the serotonergic system is the finding of Ricaurte et al. (1980) that both drugs produce a loss of synaptosomal 5-HT uptake sites.

Administration of methamphetamine to iprindole-treated rats caused decreases in both neostriatal TH activity and DA concentrations (Figure 4). Enzyme activity was decreased by 50% and DA concentrations by 73% at 3 days. No recovery was noted by 14 days in either parameter. These findings are in agreement with those of Steranka (1981) who reported that administration of a single dose of amphetamine sulfate (9.2 mg/kg) to rats treated with iprindole hydrochloride (10 mg/kg) produced marked decreases in neostriatal concentrations of DA and its metabolites. These effects were apparent by 12 hours and persisted for at least 4 weeks. Although Steranka (1981) observed significant decreases in neostriatal dopamine concentrations after 7 days using a dose of amphetamine sulfate of 9.2 mg/kg, in the study reported here a dose of methamphetamine of

17.5 mg/kg was required to produce a significant decrease. This suggests, perhaps, that the dopaminergic system in the neostriatum is more sensitive to the neurotoxic effects of amphetamine than methamphetamine.

There was a difference in the responses of the neostriatal dopaminergic and serotonergic system to the administration of iprindole and methamphetamine. One day following treatment decreases in TPH and TH activities were observed; however, neurotransmitter concentrations were only significantly decreased in the dopaminergic system. Bakhit et al. (in press) and Ricaurte et al. (1980) have shown that the chronic administration of methamphetamine produces long-lasting depletions of regional 5-HT and DA concentrations and have suggested that the serotonergic system is more sensitive than the dopaminergic system to the apparent neurotoxic effects of the drug. The results of this study indicate, however, that after single doses of the drug in the presence of a metabolic inhibitor, greater changes are observed in the dopaminergic system after 1 day. Changes in enzyme activity occur at an earlier time point in the serotonergic system than changes in the neurotransmitter concentrations whereas TH activity and DA concentrations are affected at approximately the same time point.

The effects of a single dose of methamphetamine are also different in the neostriatal serotonergic and dopaminergic systems with a significant decrease still observed in both TH activity and DA concentrations after 24 hours (Figure 4), but not in TPH activity, 5-HT or 5-HIAA concentrations (Figure 3). This suggests that the effects of the drug are more readily reversible in the serotonergic than the dopaminergic system and is supported by the earlier work of Hotchkiss and Gibb (1980) who

showed that prolonged depression of neostriatal TPH activity only occurred after 3 doses of methamphetamine, whereas TH activity was irreversibly depressed after 2 doses. The finding of a decrease of neostriatal TH activity 24 hours after a single dose is in agreement with that of Kogan et al. (1976).

The effects of iprindole alone on the serotonergic system of the cerebral cortex was unexpected. After a single injection (10 mg/kg) TPH activity was increased to 138% of saline control at 1 day and then fell to 82% of control at 3 days; 5-HT and 5-HIAA concentrations were significantly increased after 3 days (Figure 13). These parameters had recovered by 7 days. No changes were seen in Try concentrations after 1 or 3 days, nor at time points earlier than 1 day. The effects of other antidepressants on cortical TPH activity, 5-HT, 5-HIAA and Try concentrations were also examined 24 hours after a single injection (Table 5). Cloimipramine, amitriptyline and chlorpromazine acted in a similar fashion to iprindole; cloimipramine and chlorpromazine also increased 5-HIAA concentrations. Desipramine increased 5-HT and 5-HIAA concentrations, without significantly changing TPH activity. Cloimipramine was the only agent to increase Try concentrations. Fluoxetine, a selective 5-HT uptake blocker, increased 5-HT concentrations.

Other workers have reported effects of antidepressant drugs on the serotonergic system. Hussein and Goedde (1980) reported that 10 daily doses of imipramine (20 mg/kg) increased TPH activity in the rabbit mesencephalon and pons medulla, with a concomitant decrease in the Km for Try. Chlorpromazine behaved similarly to imipramine without lowering the Km value. Neckers et al. (1977) found that cloimipramine decreased midbrain Try content and increased TPH activity in rat

forebrain after a single injection. Badawy and Evans (1981) reported that a single dose (10 mg/kg) of several antidepressants inhibited rat liver tryptophan pyrrolase, the enzyme responsible for the conversion of Try to N-formylkynurenine; this results in an increase in brain Try concentrations after 3.5 hours. Although iprindole was effective in increasing brain Try concentration, chlorpromazine was shown to be ineffective.

These effects of the antidepressants may not necessarily be linked to the clinical efficacy of the drugs, as therapeutic benefit commonly takes 2 weeks to become apparent. Traditionally, it was thought that the typical antidepressants act by increasing aminergic transmission through reuptake blockade of NE or 5-HT or both. This hypothesis has been questioned because of the existence of "atypical antidepressants," such as iprindole and mianserin, which are not efficient reuptake blockers but are clinically effective, and by the fact that although these biochemical effects occur soon after the drugs are administered, the therapeutic effect requires more time to develop. More recent work has shown that treatment of rats with antidepressants for 2 weeks reduces the sensitivity of β-adrenergic receptors in brain, implying that antidepressants act be decreasing, rather than increasing, postsynaptic noradrenergic transmission. They are compatible, however, with the finding that typical antidepressants block amine reuptake. At least part of the subsensitivity produced by some of the drugs may be a consequence of reuptake inhibition, the resultant high synaptic cleft concentrations of NE would desensitize the receptor.

The action of iprindole on the cortical serotonergic system may indicate that this antidepressant, and possibly others, regulate the

neuronal conversion of Try to 5-HT. Mandell and Knapp (1977) have shown that there is a low and high affinity uptake system for Try in synaptosomal preparations from 5-HT rich areas and that the high affinity system is specific for Try. Lithium chloride induced a dose- and time-related increase in the relative velocity of high affinity uptake of Try and thus an increased conversion of Try to 5-HT. Short-term treatment increases the uptake and the capacity for intact striatal synaptosomes to convert Try to 5-HT. Long-term treatment maintains the increase in uptake and leads to a compensatory decrease in soluble enzyme activity. The interaction of the increased uptake and decreased enzyme is an eventual return to a control level. It is possible that this increased conversion is due to an increase in the brain Try concentration, a reported finding of lithium chloride administration (Fernstrom and Wurtman, 1971); however, experiments by Mandell and Knapp (1977) have indicated that at high doses of Try the intrasynaptosomal pool may be driven by whole brain concentrations and diffusion, whereas at lower levels the brain cell membrane uptake system is limiting.

It is possible that a single injection of iprindole produces changes in the high affinity uptake system resulting initially in an increased conversion of Try to 5-HT, followed by a compensatory decrease in TPH activity and eventually a return to a control situation. The finding that iprindole only alters the cortical serotonergic system is difficult to explain. Selective distribution of the drug in different brain regions may be the answer; this certainly occurs with amphetamine (Eison et al., 1981), explaining why certain regions are relatively resistant to its effects.

The effects of PCP on the neostriatal serotonergic system were not as dramatic as those of methamphetamine. The only significant changes were an increase in 5-HIAA concentrations at 15 and 30 minutes after an acute injection of 10 mg/kg (Figure 14), which may reflect an increase in release and turnover of 5-HT. An acute injection of methamphetamine (10 mg/kg) also increased neostriatal 5-HIAA concentrations soon after injection, although at later time points 5-HT concentrations were decreased. Unlike the effects of subacute and chronic methamphetamine treatment on neostriatal 5-HT and 5-HIAA concentrations, similar treatments with PCP did not alter these properties.

Cerebellar GAD activity was, however, depressed by subacute administration of PCP (Figure 15); an effect noticeable 12 hours after 4 doses of 5 and 10 mg/kg. This effect did not occur until 6 hours after the last injection and enzyme activity had recovered by 24 hours (Figure 16). These effects were not seen after both acute and chronic administration and were not apparent in other brain regions examined. This effect on GAD activity in the cerebellum may well be secondary to the actions of PCP on the noradrenergic system. Marwaha et al. (1980) have shown by electrophysiological techniques that PCP mimics the action of norepinephrine on cerebellar Purkinje cells possibly by increasing release and blocking reuptake. Stimulation of the nucleus locus coeruleus, or local application of norepinephrine, elicits a slowing of spontaneous discharge, accompanied by hyperpolarization and increased resistance of the Purkinje cell membrane (Hoffer, Siggins, Oliver and Bloom, 1973). Purkinje cell terminals contain GAD and their hyperpolarization may result in an inhibition of enzyme activity, possibly accounting for the convulsant activity of large doses of PCP seen in

some species.

In conclusion, we have demonstrated that increasing the duration of action of methamphetamine by administering iprindole as a metabolic inhibitor, produces long-term changes in the serotonergic system of the cerebral cortex, neostriatum and hypothalamus and in the dopaminergic system of the neostriatum. This study suggests that the neurotoxic effects of methamphetamine closely resemble those of the halogenated amphetamines on the serotonergic system. Injection of iprindole alone produced changes in the cortical serotonergic system, possibly reflecting a regulatory phenomenon of this antidepressant on either TPH activity or uptake of Try. This finding may be of importance in the understanding of the regulation of the 5-HT system and may also reflect subtle changes seen shortly after antidepressant therapy.

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